Advances in Experimental Medicine and Biology 863

Neville Vassallo *Editor*

Natural Compounds as Therapeutic Agents for Amyloidogenic Diseases

Springer

Advances in Experimental Medicine and Biology

Volume 863

More information about this series at http://www.springer.com/series/5584

Neville Vassallo
Editor

Natural Compounds as Therapeutic Agents for Amyloidogenic Diseases

Springer

Editor
Neville Vassallo
Department of Physiology
and Biochemistry
University of Malta
Msida, Malta

ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISBN 978-3-319-18364-0 ISBN 978-3-319-18365-7 (eBook)
DOI 10.1007/978-3-319-18365-7

Library of Congress Control Number: 2015942700

Springer Cham Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media (www.springer.com)

Preface

A large group of human diseases are characterised by the deposition of insoluble proteinaceous lesions, termed amyloid. The underlying pathogenic process in these disorders involves the misfolding and self-assembly of native monomeric proteins into toxic, multimeric aggregates. Indeed, "protein misfolding" is one of the most researched topics in *cell biology* and *sub-cellular biochemistry* in recent years. To date, more than 20 amyloidogenic proteins have been identified, including amyloid-beta and tau in Alzheimer's disease, alpha-synuclein in Parkinson's disease, huntigtin in Huntington's disease, superoxide dismutase in amyotrophic lateral sclerosis, prion protein in Creutzfeldt-Jakob disease and islet amyloid polypeptide in type 2 diabetes mellitus. Collectively, these disorders inflict an enormous economical burden on societies, whilst being devastating at a personal level. It is therefore of paramount importance to understand their origin and learn how to treat, or prevent, them. At present, there are no approved therapies that target amyloid formation directly, but a wealth of experimental and epidemiological evidence now indicates that various naturally derived compounds have beneficial anti-amyloid effects.

This book volume was written with the aim of disseminating the state-of-the-art and most recent knowledge of the field, highlighting the most promising nutra-therapeutics derived from natural compounds or extracts. The focus is mainly on Alzheimer's disease and related dementias; however, Parkinson's disease and other forms of cerebral amyloidosis are also discussed. Evidence is presented based upon published studies that have undergone rigorous scientific peer review. Researches include a combination of in vitro (biophysics, biochemistry, molecular and cellular biology), in vivo (fruit fly, transgenic mice and rats) and epidemiological (human) studies, so as to provide the reader with a holistic scientific approach. Among the therapeutic agents that are discussed in detail, we find Mediterranean diet and olive oil polyphenols, teas and catechins, natural phenolic compounds and bioflavonoids. Given the recent surge of public interest in herbal medicines, a chapter is devoted to evidence-based traditional Chinese medicine in brain ageing. Individual chapters are written by established scientists in their relevant fields; sufficient background is given in each chapter by way of introduction to the non-expert reader. Hence, this book should be of interest to a wide audience, including academia, various health professionals, the nutraceutical industry and undergraduate students reading for pharmaceutical, medical, biology and chemistry degrees.

I conclude by stating that, on a personal level, the most noble goal of this book is that with our collective contributions to scientific progress, we aim to provide hope for the future and hope for the many people suffering from as yet incurable amyloid diseases. This book is, after all, dedicated to them.

Msida, Malta Neville Vassallo

Contents

Olive Oil Phenols as Promising Multi-targeting Agents Against Alzheimer's Disease

1

Stefania Rigacci

Abstract

Amyloid diseases are characterized by the deposition of typically aggregated proteins/peptides in tissues, associated with degeneration and progressive functional impairment. Alzheimer's disease is one of the most studied neurodegenerative amyloid diseases and, in Western countries, a significant cause of dementia in the elderly. The so-called "Mediterranean diet" has been considered for long as the healthier dietary regimen, characterised by a great abundance in vegetables and fruits, extra virgin olive oil as the main source of fat, a moderate consumption of red wine and a reduced intake of proteins from red meat. Recent epidemiological studies support the efficacy of the Mediterranean diet not only against cardiovascular and cancer diseases (as previously demonstrated) but also against the cognitive decline associated with ageing, and several data are highlighting the role played by natural phenols, of which red wine and extra virgin olive oil are rich, in such context. In the meantime, studies conducted both *in vivo* and *in vitro* have started to reveal the great potential of the phenolic component of extra virgin olive oil (mainly oleuropein aglycone and oleocanthal) in counteracting amyloid aggregation and toxicity, with a particular emphasis on the pathways involved in the onset and progression of Alzheimer's disease: amyloid precursor protein processing, amyloid-beta (Aβ) peptide and tau aggregation, autophagy impairment, neuroinflammation. The aim of this review is to summarize the results of such research efforts,

S. Rigacci (✉)
Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Viale Morgagni 50, 50134 Florence, Italy
e-mail: stefania.rigacci@gmail.com

N. Vassallo (ed.), *Natural Compounds as Therapeutic Agents for Amyloidogenic Diseases*, Advances in Experimental Medicine and Biology 863, DOI 10.1007/978-3-319-18365-7_1

showing how the action of these phenols goes far beyond their renowned antioxidant activity and revealing their potential as multi-targeting agents against Alzheimer's disease.

Keywords
Alzheimer's disease • Mediterranean diet • Polyphenols • Extra virgin olive oil • Oleuropein aglycone

1.1 Introduction

Age-associated cognitive decline, in the relatively benign form of mild cognitive impairment (MCI) and in the more severe one of Alzheimer's disease (AD), has become a considerable social and clinical problem during the last decades, particularly in Western countries. With the important exception of familial AD, which is associated with one of several genetic mutations favouring the early onset of the disease, such a condition develops over a considerable period of time, maybe even decades. Moreover, in spite of the great efforts spent by researchers to identify early and accessible markers of this disease, AD is still diagnosed very late, when the neurological symptoms appear and the neuropathology is already in an advanced stage. For these reasons (life-long development – late diagnosis – neuronal loss) two important branches of the research in this field are early diagnosis and prevention.

With regards to prevention, a lot of attention has been placed on the role played by different lifestyles in favouring this disease. Epidemiological evidence points to a lower incidence of MCI and AD in populations adhering to the Mediterranean diet (MD), a dietary regimen that has already been strongly associated with a reduced risk for cardiovascular diseases and cancer (Martinez-Gonzalez et al. 2011; Sofi et al. 2010; Benetou et al. 2008; Lopez-Miranda et al. 2010). MD is characterized by extra virgin olive oil (EVOO), high intake of plant-based foods, relevant consumption of seafood, low-to moderate intake of dairy products, low intake of meat and a regular but moderate intake of red wine. In spite of regional and cultural variations (for example, fish or cheese can be more-or-less abundant), one ingredient should never be absent from the Mediterranean table: EVOO, probably the most typical component of MD. EVOO consists of 98 % glycerides, mainly monounsaturated fatty acids (MUFA). In any case, it is the remaining 2 %, including various so-called "minor compounds", that deserves much of our attention in this context. These consist of α-tocopherol and of several specific phenolic compounds including phenolic acids (caffeic, vanillic, syringic, *p*-coumaric, *o*-coumaric, protocatechuic, sinapic, *p*-hydroxybenzoic and gallic), phenolic alcohols (tyrosol and hydroxytyrosol), lignans (acetoxypinoresinol and pinoresinol), flavones (apigenin and luteolin) and, last but not least, secoiridoids (oleuropein aglycone, oleochantal and their derivatives). The latter are the most abundant and typical phenolic components of EVOO; hence investigations aimed at identifying the active principles responsible for the specific healthy benefits of EVOO have been mainly focused on them (Servili et al. 2009).

The concentration of these substances in oil is highly variable, depending on a number of different factors: olive cultivar, ripening stage at harvesting, geographic origin of olives, olive trees irrigation, operative conditions applied during crushing, malaxation and oil separation, oil storage modalities. The last two factors are particularly relevant. In fact, the highest phenolic content is present in oil immediately after its cold mechanical extraction from olives (a mandatory procedure to obtain an EVOO) and progressively declines with oil ageing, particularly if it is exposed to air and light that promote phenols oxidation and degradation. With some notable exceptions, total phenols in olive oil generally

range between 130 and 350 mg/kg, with EVOO at the highest end of this concentration interval.

Regarding the protection provided by EVOO consumption against age-associated cognitive decline, the results provided by the studies on the PREDIMED (PREvención con DIeta MEDiterránea) cohort are particularly interesting. The aim of these clinical trials was to test the efficacy of MD in counteracting cardiovascular disease events in asymptomatic people at high cardiovascular risk. In one of these studies, 578 subjects reasonably conforming to the traditional MD (except for a higher intake of meat and diary products) were evaluated for their cognitive performance and total urinary polyphenol excretion (Valls-Pedret et al. 2012). Results showed that higher intake of both total olive oil and its virgin variety, coffee, walnuts, and wine were associated with better memory function and global cognition. Interestingly, the consumption of different foods and beverages was found significantly associated with the improvement in specific cognitive capabilities. Thus, EVOO and coffee were found to associate with better delayed verbal memory, walnuts with improved working memory, and red wine with higher Mini-Mental State Examination scores. Urinary phenols excretion was dose-dependently associated with improved memory, supporting the hypothesis that the common denominator of all those different foods, that is their high phenolic content, was the main factor responsible for the observed benefits.

The more recent PREDIMED-Navarra randomized trial included a cohort of 268 subjects (74.1 ± 5.7 years old, 44.8 % men with no cardiovascular disease but at high vascular risk because affected by type 2 diabetes mellitus or by three or more vascular risk factors) which were randomly assigned to three groups receiving for 6.5 years a low-fat diet (control), or a typical MD containing either EVOO (1 L/week) or mixed nuts (30 g/day) (Martinez-Lapiscina et al. 2013). The intervention with the EVOO-diet was associated with better cognitive performances, especially across fluency and memory tasks, and less MCI as compared to controls, while no significant differences were associated with the nuts-diet. Moreover, those who received the EVOO supplementation had significantly better performances on both visual and verbal memory domains compared to those who received nuts supplementation. These results support an inverse association between the consumption of EVOO and amnestic cognitive impairment, the deficit most commonly associated with the risk for AD. These data further suggest that, in contrast to what was postulated to justify the reduced cardiovascular disease risk associated with MD, it was not the unsaturated lipid component of EVOO that was the major determinant factor for its beneficial effect in this context; indeed, nuts are a valuable source of polyunsaturated fatty acids too. These and previous data strongly indicate that EVOO phenolic content is the main ingredient responsible for the reduced risk for age-related cognitive impairment (Jacomelli et al. 2010).

But what exactly is the mechanism by which EVOO phenols exert their protection? These molecules have for long been considered just for their antioxidant activity (Tasset et al. 2011; Pierno et al. 2014; Farr et al. 2012) but accumulated data now suggest that other properties play an even more important role when we look at the maintenance of a good cognitive performance. For example, recent research conducted on C57Bl/6 J mice fed with an EVOO rich in phenols (10 % EVOO weight/weight of dry diet for a daily total polyphenol dose of 6 mg/kg), showed improved contextual memory and reduced age-related impairment in motor coordination with respect to controls. While the latter effect was associated with reduced lipid peroxidation in the cerebellum, the former did not correlate with oxidation or inflammation parameters, suggesting the involvement of other mechanisms not relying on increased antioxidant protection (Pitozzi et al. 2012). In support of this, are studies on the determination of the antioxidant activity of the main phenolic derivatives in human biological fluids after dietary EVOO intake, i.e. hydroxytyrosol (HT), tyrosol and homovanillyl alcohol glucuronide derivatives, at concentrations that covered all biologically relevant ranges (0.01–0.1 μM). The results of such assays show that glucuronidation rapidly decreases the protective activity of

EVOO phenols against Cu-mediated low-density lipoprotein (LDL) oxidation and in 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, confirming that the biological activity of a phenol-rich olive oil cannot merely be an antioxidant one (Khymenets et al. 2010).

Actually, the investigation on individual EVOO phenolic compounds has uncovered a lot of specific biochemical activities in different biological contexts. Here I will review the most relevant ones for what concerns AD counteraction, but first of all I will provide a brief description of this disease, particularly for those that are not completely familiar with its biochemical traits.

1.2 General Traits of Alzheimer's Disease

AD is characterised by a progressive decline of several cognitive domains including memory, visuospatial skills and executive function (Sa et al. 2012). The histopathological analysis of affected brains reveals selective neuronal degeneration and synaptic loss, particularly in the hippocampus, amygdala and temporal neocortex (Serrano-Pozo et al. 2011), accompanied by extracellular senile plaques and intraneuronal neurofibrillary tangles. The main constituent of plaques is a fibrillar network of polymeric unbranched fibrils made of Aβ peptides, fragments of different length; the most represented are Aβ(1–40), Aβ(1–42) and the highly aggregation prone N-terminally truncated Aβ(3–42) and Aβ(11–42) pyroglutamylated-species. Aβ fragments originate from the sequential activity of two proteases, γ-secretase and β-secretase (BACE) that act on the extracellular N-terminal domain of the transmembrane amyloid precursor protein (APP). These natively unfolded peptides aggregate into increasingly ordered structures (oligomers – protofibrils – fibrils) that become insoluble and resistant to the clearing activities of cells and tissues (Mohamed et al. 2011), thanks to the acquisition of a peculiar β-sheet rich fold called "amyloid structure" (Fig. 1.1) (Serpell et al. 2000).

Neurofibrillary tangles are amyloid in nature too, being mainly formed by the cytoskeletal protein tau, a member of the microtubule-associated protein family normally concurring to the assembly and stabilization of microtubules, physiologically involved in axonal transport and neurite outgrowth (Maccioni et al. 2001). Tau exists in six different isoforms (45–65 kDa) originating from alternative splicing; it is a hydrophilic cationic protein, unfolded under native conditions (Jeganathan et al. 2008), whose normal function is regulated by phosphorylation, glycosylation, ubiquitination, truncation, and nitration (Farias et al. 2011). In AD, abnormal phosphorylation occurs on specific tau residues (Ser202, Thr205, Ser235, and Ser404) (Alvarez et al. 1999). These post-translational modifications are catalyzed by two main protein kinases: the Cyclin-dependent kinase (Cdk)5/p35 system and glycogen synthase kinase-3 beta (GSK-3β) (Farias et al. 2011), and are thought to promote tau aggregation.

More than two decades ago, Hardy and Higgins (1992) put forward the "amyloid cascade hypothesis" of AD, posing the insoluble Aβ fibrils as the primary toxic species. More recently, widespread support has been provided for the "toxic oligomer hypothesis" which proposes that pre-fibrillar intermediates are the toxic determinant of several amyloid diseases, including AD (Benilova et al. 2012; Brorsson et al. 2010; Walsh et al. 1999, 2002; Walsh and Selkoe 2004; Cleary et al. 2005; Billings et al. 2005; Koffie et al. 2009). Accordingly, such oligomers have been retrieved in diseased tissues both from animal models and humans, and *in vitro* assays have repeatedly confirmed their toxicity to cells. Several mechanisms determine oligomer cytotoxicity: membrane destabilization and derangement of ion homeostasis, particularly of Ca^{2+}; oxidative damage; overload and dysfunction of the pathways devoted to protein quality control (proteasome, unfolded protein response, autophagy). With regards to tau, its oligomers are also now considered to be the most toxic species, possibly inducing neurodegeneration by affecting mitochondrial and synaptic function, both of which are early hallmarks of AD

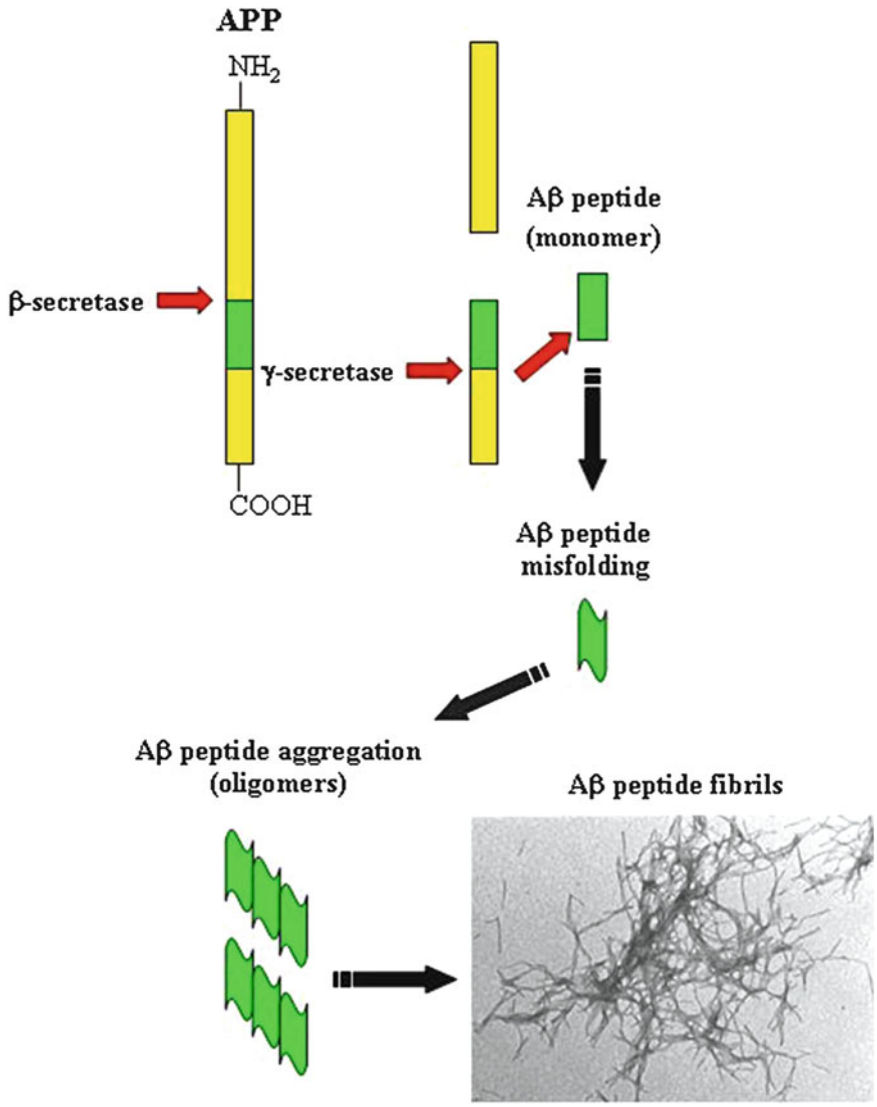


Fig. 1.1 Aβ peptide generation from APP by γ-secretase and β-secretase, followed by aggregation into oligomers and fibrils

and other tauopathies (Lasagna-Reeves et al. 2011; Guzman-Martinez et al. 2013).

Recently, the complexity of the amyloid scenario has been further increased by the discovery of different species of oligomers, varying in their structural features as well as in their toxicity (the so-called "amyloid polymorphism") (Lee et al. 2007; Meinhardt et al. 2009; Stefani 2012). Moreover, a reappraisal of the role of fibrils in amyloid toxicity is taking place in recent years, particularly concerning AD, supporting a role for Aβ fibrils not only as a possible reservoir of toxic oligomers but also as a neurotoxic species in their own right (Pan et al. 2011; Gilbert 2013).

As efficaciously summarized in a recent review, inflammation seems to play an important role in AD pathogenesis (Meraz-Rios et al. 2013). While initial activation of microglia and particularly astrocytes may be a positive response in the attempt to clear protein aggregates through phagocytosis and intracellular degradation, this process soon becomes dysfunctional leading to a

worsening of the pathology through the production of nitric oxide (NO), reactive oxygen species (ROS), pro-inflammatory cytokines (tumor necrosis factor-α, interleukin-1β and interleukin-6), and prostaglandin-E2, which eventually promote neuronal death. Moreover, pro-inflammatory cytokines mediate an increase in tau phosphorylation, APP synthesis and Aβ generation through BACE-1 transcription (Lyman et al. 2014; Chen et al. 2012; Krstic et al. 2012).

This brief description of AD, which does not pretend to be exhaustive, can give an idea of the complexity of this pathology; accordingly, the search for possible therapeutic interventions must face such a highly multiform scenario. The investigation of EVOO phenols in the context of amyloid diseases has started relatively later with respect to other extensively-studied polyphenols like curcumin, epigallocatechin-gallate (EGCG) and resveratrol. Nevertheless, the results obtained with oleuropein aglycone (OLE) and oleocanthal (OLC) are promising. Here, I will critically review published reports, placing an emphasis on the multi-targeting potential of such compounds which, in my opinion, constitutes their main strength.

1.3 Oleuropein Aglycone

OLE or 3,4-dixydroxyphenylethanol elenolic acid (3,4-DHPEA-EA) (Fig. 1.2), is a secoiridoid derived from oleuropein by the activity of a β-glucosidase released from olive fruits during crushing (Brenes et al. 1999). Its content in EVOO is highly variable, depending on several factors: olive cultivar (Romani et al. 1999; Franconi et al. 2006), ripening (Brenes et al. 1999), method of oil production (Tripoli et al. 2005) and storage conditions, with oxygen and light being the most detrimental factors by promoting oxidation (Cicerale et al. 2009). Moreover, the method of extraction of OLE from oil samples and the procedure followed to determine its concentration seem to considerably affect the reported values. To give a general idea, I will refer in this review to the work of Servili *et al.* who analyzed 263 EVOO samples and found

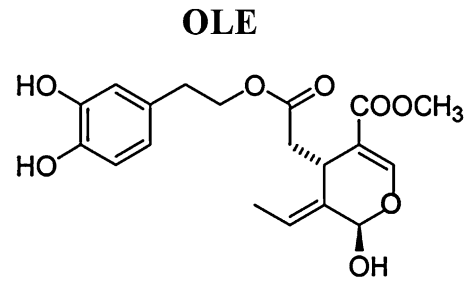


Fig. 1.2 Oleuropein aglycone (OLE)

a median value of 137 mg/kg for OLE and of 308 mg/kg for its dialdehydic form 3,4-DHPEA-EDA, also called Oleacein (Servili et al. 2009). Multiple beneficial effects were demonstrated for OLE, ranging from anti-atherogenic and anti-hypertensive to anti-cancer, anti-microbial and anti-inflammatory (Rigacci and Berti 2009) and in the last years promising anti-amyloid, neuroprotective and gerosuppressant activities have started to emerge for this secoiridoid.

Preliminary Mass Spectrometry (MS) analysis performed on its glycoside, oleuropein, revealed that it can associate the monomeric form of $A\beta 1-40$ and the oxidized form $A\beta Met^{35}(O)$ with a 1:1–2:1 oleuropein:peptide stoichiometric ratio. Such interaction is non-covalent but possesses a remarkable binding energy, since the complexes are still observable when an orifice potential of 100 V is applied to the Electrospray ionization (ESI)-MS apparatus (Bazoti et al. 2006, 2008). Through enzymatic cleavage of the Aβ:oleuropein complex prior to ESI-MS analysis, three peptide regions were identified as being implicated in the interaction between the two and, of these, the hydrophobic (17–21) one was the best candidate for the association with the non-polar moiety of oleuropein and hence also with its aglycone derivative OLE (Galanakis et al. 2011). The importance of this peptide region for the interaction of Aβ with oleuropein has been further confirmed using NMR (Benaki et al. 2009; Kallberg et al. 2001). Interestingly, the Aβ sequence critical for peptide fibrillization overlaps the putative OLE-binding region (Kallberg et al. 2001; Tjernberg et al. 1996, 1999);

thus, an interference of such secoiridoid with peptide aggregation could somehow be anticipated.

Actually, most of the studies on oleuropein as an aggregation inhibitor were performed on its aglycone OLE, which was shown to interfere with both Aβ42 and human islet amyloid polypeptide (hIAPP) aggregation *in vitro* (Rigacci et al. 2010, 2011). Through a combination of structural analysis by Thioflavin-T (ThT) and Anilinonaphthalene-8-sulfonate (ANS) binding, Circular Dichroism (CD) analysis, Atomic Force Microscopy (AFM), Transmission Electron Microscopy (TEM), and toxicity assays on cultured cells, it was demonstrated that OLE is capable of redirecting the aggregation pathway, thereby avoiding the formation of toxic oligomers and triggering peptide precipitation into amorphous aggregates from which non-harmful protofibrils eventually evolve. Concerning hIAPP, the amorphous aggregates that originate during the first phases of incubation in the presence of OLE are unable to interact with, and to damage, the cell membrane (Rigacci et al. 2010). Pre-existing Aβ fibrils can also be remodeled by the addition of OLE, with a reduction in the density of the fibrillar deposit and, most importantly, no release of toxic fragments (Rigacci et al. 2011).

Such preliminary *in vitro* results paved the way to experimentation on model organisms, which have corroborated the idea that OLE can be protective against amyloid aggregation and aggregate toxicity not only in cultured cells but also *in vivo*. In fact, administration of OLE to the CL2600 *Caenorhabditis elegans* (*C. elegans*) strain, constitutively expressing Aβ(3–42), resulted in significantly lower plaque deposition and toxic oligomer formation, with a reduction in the extent of worm paralysis and an increase in survival (Diomede et al. 2013). When Aβ(1–42) was aggregated in the presence of OLE and then injected in the *nucleus basalis magnocellularis* of adult male Wistar rats, it was not toxic to cholinergic neurons and did not raise an inflammatory reaction. This is in contrast to what happened when Aβ aggregates, grown under the same conditions but in the absence of OLE, were injected. The much lower amount of soluble toxic oligomers (recognized by the conformation-specific A11 antibody) in the injected rat brain suggested that OLE stably hinders the formation/release of toxic species, also when the aggregated peptide is introduced in a complex tissue environment (Luccarini et al. 2014).

A significant step forward in the *in vivo* research on the anti-amyloid potential of OLE was made by using the TgCRND8 transgenic mouse model of AD, encoding a double-mutant form of APP and showing cognitive impairment and amyloid plaque deposition from the age of 3 months. A robust improvement in cognitive performance and a remarkable reduction in Aβ plaque number, size and compactness was evident in mice fed for 8 weeks with an OLE-supplemented diet (50 mg/kg of diet). Improvement occurred even when the treatment was started at 4 months, when amyloid deposits are already present, hence suggesting that OLE can not only prevent amyloid deposition but also disaggregate preformed plaques. In this model, other relevant biological effects of this secoiridoid were uncovered: microglia migration to the plaques for phagocytosis was increased, astrocyte reaction was reduced, hippocampal neurogenesis was stimulated and, most interestingly, an intense and functional (i.e. leading to substrates degradation following vacuoles fusion with lysosomes) autophagy induction was elicited. Indeed, data obtained with cultured N2a mouse neuroblastoma cells confirmed that OLE dose-dependently activates autophagy acting on the "classical" pathways which involves mTOR inhibition (Grossi et al. 2013, 2014).

Notably, OLE antioxidant activity does not seem to be relevant in these systems. In fact, OLE does not significantly reduce either lipid peroxidation in the cortex of TgCRND8 mice (Grossi et al. 2013), nor intracellular superoxide level in the transgenic CL2600 *C. elegans* strain (Diomede et al. 2013). Rather, OLE acts both as an inhibitor of amyloidogenic peptide aggregation (possibly through direct binding, as suggested by the previously cited MS and NMR studies) and as a signaling molecule promoting cellular protective responses like autophagy stimulation and inflammation reduction. The latter property sounds particularly attractive since

the pathogenetic role of neuroinflammation in AD seems relevant – perhaps, even more so than the role of amyloid toxicity (McGeer and McGeer 2013). Though this position is debatable, it is a fact that AD progression is accompanied by a widespread neuroinflammation. Accordingly, incidence-based, population-based and case-control studies in humans suggest that some non-steroidal anti-inflammatory drugs (NSAIDs) could provide a degree of protection against AD (Vlad et al. 2008; in t' Veld et al. 2001). In any event, the ineffectiveness of different NSAIDs in several clinical trials leads us to hypothesise that, maybe, they work as preventative agents only if their administration is started early on – at least 2 years before the clinical diagnosis of AD (McGeer and McGeer 2013). This, together with the adverse gastrointestinal and cardiovascular effects that were recorded in some clinical trials, strongly reduce the potential of NSAIDs as anti-AD drugs (Scharf et al. 1999; Group 2006). Also for these reasons, research on the anti-inflammatory properties of natural compounds is particularly active and has led to substantiate at molecular level the already documented efficacy of EVOO and of its phenolic component in this respect. For example, significant reductions in serum leukotriene B4 and thromboxane B2 concentrations at 2 and 6 h after consumption of EVOO, but not after consumption of either olive oil or corn oil (both poor in phenols) were observed (Bogani et al. 2007); a 3-month intervention with a MD characterised by EVOO as the main source of fats determined a decrease in the expression of inflammation related genes (INF-γ, Rho GTPase-activating protein-15 and interleukin-7 receptor) higher than that coming from the adoption of a MD containing an olive oil poor in phenols (Konstantinidou et al. 2010); 50 μM OLE (a concentration that was supposed to be reached in plasma by individuals consuming an EVOO-rich diet) inhibited tumour necrosis factor-α (TNF-α) induced matrix metalloproteinase 9 (MMP-9) expression and secretion in THP-1 human monocytic leukaemia cells by impairing NF-κB -mediated genes transcription (Dell'Agli et al. 2010); similar oleuropein and HT concentrations inhibited PMA-stimulated COX-2 and MMP-9 expression and activities thus reducing inflammatory angiogenesis in cultured endothelial cells (Scoditti et al. 2012); in this case too, a reduction in the ROS-sensitive NF-κB transactivation was observed. However, the opposite result was obtained in a different cell type (embryonic kidney HEK293 cells stably expressing APP) where oleuropein seemed to increase MMP-9 gelatinolytic activity in the culture medium (Kostomoiri et al. 2013). The authors observed that treatment with oleuropein modified APP processing, increasing the formation of the non-amyloidogenic and neuroprotective sAPPα fragment, and they attributed this result to an increase in the α-secretase activity of MMP-9. The discrepancy between the two studies could derive from the different conditions employed (different cell lines, PMA-stimulated or basal MMP-9 secretion) and the different approaches to MMP-9 evaluation (in the first case MMP-9 mRNA level, protein expression and enzymatic activity were evaluated and found to be decreased (Scoditti et al. 2012)) while in the second study only MMP-9 gelatinolytic activity was determined (Kostomoiri et al. 2013). OLE dose-dependently inhibited the production of the pro-inflammatory chemokine CCL2 by human endothelial cells too, thus reducing endothelium inflammation (Sindona et al. 2012), and attenuated the inflammatory response in a carrageenan-challenged mouse model of inflammation (Impellizzeri et al. 2011).

The potential of OLE in the context of AD is corroborated also by its ability to inhibit the fibrillization of tau (both wild-type and carrying the P301L mutation, a mutation that increases its aggregation propensity and leads to frontotemporal dementia and parkinsonism in carriers) leading to reduced deposition of fibrillar material in the form of short rods (TEM analysis) (Daccache et al. 2011). The IC50 of OLE for the inhibition of P301L tau aggregation was 1.3 μM, as determined by the Thioflavin-S (ThS) fluorescence assay; in contrast, HT was poorly effective, inducing the growth of fibrils that were less dense but structurally similar to those originated in the absence of any inhibitor, thus confirming the

superiority of the secoiridoid as an anti-amyloid agent. Data concerning the toxicity of tau aggregates obtained in the absence or in the presence of OLE are still lacking.

Olive oil phenols are renown for their antioxidant activity. Nevertheless, the relevance of this property following ingestion is questionable because of phenols modification (this point will be more thoroughly discussed in Sect. 1.4). Nevertheless, an increase in cellular antioxidant defense can be observed following exposure to these molecules: a 6-month oleuropein administration to aged rats (50 mg/kg body weight/day) significantly increased superoxide dismutase, catalase and glutathione peroxidase activities in the brain, thus reducing the lipoperoxidative damage. Interestingly, a concomitant increase in dopaminergic neurons in *substantia nigra* was also observed. This could derive from the increase in enzymatic antioxidant defense, since these neurons are particularly sensitive to oxidative stress because of their abundance in iron ions and in free radicals, generated during dopamine metabolism (Sarbishegi et al. 2014).

With regards to the molecular mechanisms by which OLE produces its cellular effects, these are far from being clearly defined. A step forward in this direction was made by investigating the whole-genome transcription profile of human breast cancer cells exposed for 6 h to an EVOO extract containing 25 % OLE and 49 % its dialdehydic form 3,4-DHPEA-EDA (Menendez et al. 2013). This extract induced a marked increase in the expression of several genes; bioinformatic analysis of the global transcriptomic profiles revealed the induction of the endoplasmic reticulum (ER) stress chaperones and of the unfolded protein response (UPR), both relevant against the accumulation of protein aggregates. Age-related changes in cell size, morphology and senescence associated β-galactosidase (SA-β-gal) staining were significantly counteracted by the EVOO extract, and these effects correlated with the increase in the expression of the histone deacetylase Sirtuin1 and the activation of the AMP-activated protein kinase (AMPK). These two proteins regulate a multitude of signaling pathways in cells and one of them, significantly involved in the maintenance of cellular homeostasis, is autophagy. AMPK can activate autophagy by both positively regulating the Atg1/(ULK1) complex and inhibiting mTOR (Cai et al. 2012), while Sirtuin1 can deacetylate and activate the pro-autophagic Atg5, 7, 8 proteins and the LKB1 kinase, which in turn can activate AMPK (Chung et al. 2010). AMPK activity deregulation in AD seems to be involved in the perturbed brain energy metabolism, in Aβ generation and accumulation and in altered tau phosphorylation (Cai et al. 2012). Although the precise mechanism by which OLE activates autophagy still needs to be defined, these pieces of evidence reporting AMPK activation by a phenol-rich EVOO extract support the hypothesis of an involvement of this pathway.

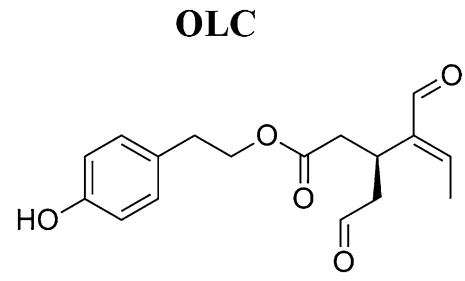


Fig. 1.3 Oleocanthal (OLC)

1.4 Oleocanthal

OLC (3,4-HPEA-EDA) is the dialdehydic form of the decarboxymethyl derivative of ligstroside aglycone 3,4-hydroxyphenylethanol elenolic acid (3,4-HPEA-EA) (Servili et al. 1999) (Fig. 1.3). The median value of its concentration in EVOO, according to Servili et al. (2009), is 85 mg/kg depending on several variables (Brenes et al. 2000, 2001; Tovar et al. 2001), as already mentioned for OLE.

Regarding OLC effects on amyloid aggregation, currently available data support its ability to convert peptide monomers and oligomers (but not fibrils) into high-molecular-weight (HMW) species. Regarding Aβ(1–42), the latter's immunoreactivity to the conformation-specific an-

tibody NU1 (recognizing oligomers) as well as to the sequence-specific M89, 4G8, and 6E10 antibodies, increased when it was incubated in aggregation conditions together with OLC. This suggests the adoption of a different conformation, while no effect was evident when OLC was added to pre-formed fibrils (Pitt et al. 2009). Such an outcome could be beneficial only if the newly-formed HMW species lost the potential to induce cytotoxic effects. The ability of OLC-modified aggregates to bind the synapses of hippocampal neurons was not significantly altered (a reduction was observed, but it was not statistically relevant); however, unfortunately, cytotoxicity of the OLC-modified aggregates was not assessed by the authors. For such reasons, these data do not, as yet, convincingly support a beneficial effect of OLC in redirecting Aβ aggregation. However, something different occurs if neurons are pre-treated with OLC before coming in contact with toxic Aβ oligomers: in such situation, a reduced binding of oligomers to the cell membrane and a protection against synaptic deterioration was observed (Pitt et al. 2009). Moreover OLC, once present in the culture medium, increases the immunoreactivity of subsequently added toxic oligomers, making them more prone to clearance by NU1 antibodies.

Collectively, these data suggest a complex interaction of OLC with amyloid peptides, with structural remodeling (and subsequently altered immunoreactivity) varying depending on the preliminary aggregation state of the amyloid species. Such preliminary pieces of evidence lead us to hypothesize that OLC could work as a preventative agent since its presence in the cellular environment prior to the appearance of amyloid deposits seems particularly efficient in inhibiting the toxic outcome. An *in vivo* experimentation is required to assess OLC actual potency in reducing toxic amyloid deposition in the brain.

Conversion of monomers and oligomers into non-fibrillar HMW aggregates was also shown for tau in the presence of OLC (Li et al. 2009). An interesting comparison of the different chemical behavior of OLE and OLC as tau aggregation inhibitors was made by Daccache et al. (2011) also on the basis of other data (Li et al. 2009; Monti et al. 2011): a structure–activity relationship study based on a series of derivatives of OLC pointed to an anti-fibrillization pharmacophore comprising both the saturated and unsaturated aldehyde moieties. Such a dialdehyde was proposed to cross-link two lysine residues within the third repeat of tau that would initiate the fibrillization process, rapidly followed by the modification of a single lysine, thus producing a cyclic adduct that evolves towards a more stable pyridinium-like complex by rearrangement of the skeleton. This would lock tau into a random coil conformation, preventing the transition to the β-sheet rich amyloid form and thus favoring the formation of non-amyloid HMW aggregates (Li et al. 2009; Monti et al. 2011). On the contrary, the methoxycarbonyl group of OLE increases the acidity of the hydrogen on the adjacent carbon thus inducing an intramolecular rearrangement leading to the dihydropyran form as the main isomer of OLE in solution. This form is in equilibrium with a monoaldheydic one that, when reacting with a lysine residue, would yield an aliphatic Schiff base that is unstable in aqueous media. OLE, therefore, does not work as a stable crosslinker and it does not promote the precipitation of tau into amorphous macro-aggregates; rather it favours the maintenance of a soluble form of tau so that the amount of fibrillar species is highly reduced (Daccache et al. 2011).

Although OLC does not seem to significantly affect the ability of tau to promote microtubule assembly in an *in vitro* tubulin polymerization assay (Li et al. 2009), some concerns still remain about retention of this important tau physiological function following OLC crosslinking, and an *in vivo* confirm is warranted.

Apart from its interference during amyloid aggregation, OLC usefulness against AD was recently supported by additional findings. A study conducted on cultured bEnd3 mice brain endothelial cells and C57BL/6 mice suggests that intraperitoneal administration of OLC to mice can increase the clearance of ^{125}I-Aβ(1–40) from the brain by up-regulating both Aβ degrading enzymes (Neprilysin and Insulin degrading enzyme) and Aβ transporters (P-glycoprotein and LDL receptor related protein-1) at the Blood

Brain Barrier (BBB) (Abuznait et al. 2013). These effects are highly relevant because the expression of such transporters declines with ageing and in AD, possibly contributing to amyloid accumulation in the brain parenchyma (Silverberg et al. 2010; Vogelgesang et al. 2004). OLC was also found to activate AMPK (Khanal et al. 2011). As already mentioned in the previous section, this kinase not only represents a node in the complex network regulating energy supply to the cell, but also participates in the quality control of proteins and organelles *via* autophagy. Autophagy is particularly beneficial and overexploited during neurodegenerative diseases, where protein amyloid deposition eventually overwhelms the cellular buffering capacity. The possibility that OLC activates autophagy, and the relevance of this in the context of AD models, has not been directly investigated yet. Last but not least, OLC possesses a striking ibuprofen-like activity: it dose-dependently inhibits COX-1 and COX-2 but has no effect on lipoxygenase *in vitro* (Beauchamp et al. 2005), and it decreases lipopolysaccharide-induced nitric oxide synthase in chondrocytes (Iacono et al. 2010). This strong anti-inflammatory activity is probably, at the moment, one of the most appealing properties of OLC, potentially useful also in counteracting AD-associated neuroinflammation.

1.5 Bioavailability

Bioavailability of phenolic compounds represents a critical issue that has to be addressed by answering three fundamental questions: (1) to what extent are these compounds absorbed following ingestion? (2) How are they modified following ingestion? (3) How do they (or their modification products.) distribute in tissues? Unfortunately these topics have been to date investigated with very different and non-standardized approaches, as it has been outlined in a recent review (D'Archivio et al. 2010), so that it is not easy to derive conclusive answers.

Concerning the phenolic compounds OLE and OLC, the main variables are represented by the form in which they are ingested (pure compounds, extracts containing different percentages of the compounds, whole EVOOs with different phenolic composition), dosing, duration of the treatment, and association with different foods. Moreover, the great majority of the studies have been performed employing oleuropein, which can be extracted from olive leafs or olive mill wastewater and so is more convenient (and commercial) than OLE, which is enriched in EVOO.

In spite of all these variables, a general consensus has emerged regarding the fact that EVOO phenols are in fact absorbed and metabolized by humans, because their degradation and modification products are retrieved in urine following ingestion (Vissers et al. 2002; Weinbrenner et al. 2004; Miro-Casas et al. 2003). Nevertheless, absorption profiles vary depending on the source of such phenols: when they are ingested as an oleuropein-rich olive leaf extract, mainly sulfate and glucuronide derivatives are detected both in plasma and in urine, with HT glucuronide being the most abundant one, hence suggesting extensive degradation of the secoiridoid component (Garcia-Villalba et al. 2014; de Bock et al. 2013). On the other hand, when phenols are introduced with EVOO, OLE, ligstroside aglycone and their Phase II metabolites are mostly abundant in urine (Garcia-Villalba et al. 2010; Suarez et al. 2010). These results support the view that secoiridoids aglycones are better absorbed than their glycated counterparts and suggest that EVOO matrix can contribute to phenols stability in the gastrointestinal tract and favour their absorption. Indeed, when EVOO is mixed with acidified water (pH 2.0) in a 1:1 ratio at 37 °C (a condition simulating the stomach environment), the secoiridoid aglycones present remain stable for 4 h (Romero et al. 2007). Under these conditions, 50 % of total phenols diffuse from EVOO into the simulated gastric juice; this percentage increases as the pH rises, whilst the EVOO:water ratio decreases to 1:2 (the latter two conditions mimic those present in the duodenum).

Oleuropein is far more stable, both in the stomach and in the intestine (over 12 h), if it is

ingested with a meal (Markopoulos et al. 2009). Conversely, oleuropein is strongly hydrolyzed in the upper gastrointestinal tract, with the rapid appearance of HT glucuronide in the plasma, when it is taken as an olive leaf extract under fasting conditions (Garcia-Villalba et al. 2014). Moreover, OLE is absorbed more efficiently than oleuropein, probably because its greater apolarity favours passive transport across the cell membrane. In fact, when rabbits are fed an EVOO containing a 9-fold concentration of OLE *vs* oleuropein, a 60-fold concentration of OLE *vs* oleuropein is reached in the plasma (Coni et al. 2000). A recent report further shows that, after oleuropein administration to rats, OLE is retrieved both in faeces and urine (together with hydrolysis and modification products) (Lin et al. 2013). Particularly relevant in the context of AD is experimental evidence suggesting that, in rat and humans, orally-administered olive oil phenols, including OLE, oleuropein and/or one of its derivatives arising from tissue metabolism, cross the BBB and are found inside brain parenchyma (Serra et al. 2012; Vissers et al. 2002). Finally, OLE and 3,4-DHPEA-EDA seem to associate to membranes as a consequence of their hydrophobicity (Paiva-Martins et al. 2010); this implies that they may accumulate at the cellular level, reaching a local concentration higher than that expected on the basis of their plasma concentration alone.

Considering that HT and tyrosol are the main degradation products of OLE and OLC, respectively, either in oil (increasing with EVOO ageing) or in the organism following ingestion, a few words should be spent on these phenols too. HT penetration into the brain has been demonstrated in a pharmacokinetic study assessing the metabolic fate of intravenously injected [^{14}C]HT (D'Angelo et al. 2001). Anti-atherogenic, anti-inflammatory, anti-microbial, anti-proliferative and pro-apoptotic effects have been attributed to HT (Granados-Principal et al. 2010). The antioxidant potency of HT *in vitro* is very high but its biological importance as a ROS scavenger has been questioned of late because this seems to be greatly affected by HT modification (i.e. glucuronidation) following ingestion (Khymenets et al. 2010). Nonetheless, when mice are fed for 12 days with 100 mg of HT/kg of body weight, basal and Fe^{2+}-induced malondialdehyde (MDA) formation are significantly reduced in explanted brain cells, in spite of no systemic increase in antioxidant capacity. This suggests that, besides direct ROS and iron-scavenging activity of HT, cytoprotection would derive also from different mechanisms (Scharf et al. 1999). Indeed, several experiments have shown that HT increases the expression of antioxidant enzymes, an ability potentially relevant in the context of AD. This happens as a result of a hormetic mechanism by which HT, behaving like a mild pro-oxidant, induces the activation of cellular defenses: in fact, in the presence of peroxidases, HT can undergo a catechol-semiquinone-quinone redox cycling generating superoxide, which in turn increases MnSOD expression. As a result, age-associated mitochondrial ROS accumulation is counteracted and the chronological lifespan of normal human fibroblasts is extended (Sarsour et al. 2012). Hormesis seems to be implied also in the increase in lifespan and stress resistance of tyrosol-fed *C.elegans*, subsequent to the activation of components of the heat shock response and the insulin pathway (Canuelo et al. 2012). The up-regulation of several antioxidant enzymes was also observed in keratinocytes exposed to HT: glutaredoxin, thioredoxin reductase, glutathione peroxidase-3, heme oxygenase-1, biliverdin reductase and ferritin, the latter three participating in the degradation of heme to biliverdin (a potent antioxidant) and in storage of the pro-oxidant free iron which is produced as a heme degradation by-product (Rafehi et al. 2012). Interestingly, in vascular endothelial cells the HT-mediated up-regulation of catalase expression and the associated protection against ROS increase are dependent on the activation of the AMPK-FOXO3a pathway, since they are abrogated when AMPK expression is suppressed by siRNA (Zrelli et al. 2011). On the basis of such HT-mediated AMPK activation we could speculate whether HT is an autophagy activator, too. However, the available data seem suggest the opposite: exposure of prostate cancer PC-

3 cells to 80 μM HT resulted in a significant increase in superoxide production accompanied by a defect in autophagy (Luo et al. 2013), while HT supplementation to rats counteracted autophagy activation induced by intense physical exercise (Feng et al. 2011). In a context closer to AD, HT was found to reduce the cytotoxicity of Aβ(25–35) to N2a neuroblastoma cells by decreasing NF-κB nuclear translocation and cell death, but no effect was observed on Aβ − or H_2O_2-induced decrease in cellular glutathione (GSH) (St-Laurent-Thibault et al. 2011). Limited data are available concerning HT activity as an amyloid aggregation inhibitor: as already mentioned, the *in vitro* aggregation of tau was affected by HT (as demonstrated by the reduction in ThS binding) but amyloid fibrils structurally similar to those grown in the absence of the phenol were eventually formed (Daccache et al. 2011).

From these data the superiority of OLC and OLE, with respect to HT, as potential drugs against amyloid diseases seems evident. Accordingly, studies are currently under way to improve the bioavailability and preserve the integrity of such secoiridoids following ingestion. A promising approach is represented by encapsulation: simple olive oil phenols (tyrosol, HT, homovanillic acid, 3,4-dihydroxyphenylacetic acid, and protocatechuic acid) have been successfully enclosed into the hydrophobic cavity of β-cyclodextrin (Rescifina et al. 2010). Oleuropein, too, was found to enter the β-cyclodextrin particle with its phenolic portion, and this seemed to protect it from oxidation and to increase its aqueous solubility (Mourtzinos et al. 2007). Further suggestions come from trials performed with other polyphenols: for instance, promising results were obtained when curcumin was encapsulated in polyethylene glycol (PEG) nanoparticles stabilised with β-cyclodextrin. Such nanoparticles (<80 nm in size) did not tend to aggregate, were fairly stable and performed well both in an *in vitro* cell monolayer permeability assay (mimicking BBB transit) and after oral administration to TgCNRD8 mice, allowing an increase in curcumin penetration into the brain (Cheng et al. 2013).

1.6 Conclusions and Perspectives

The beneficial effects of MD in counteracting human diseases and aging are widely recognised, and multiple evidence points to EVOO as one of the most valuable ingredients of such a dietary regimen. The main differences in the composition of EVOO with respect to other edible oils are the prevalence of oleic acid as the main lipid (up to 85 % of total fatty acids, as compared with 14 to 59 % in sunflower, soybean and peanut oils) and the minor unsaponifiable fraction (0.5–2 %) containing squalene, tocopherol, various sterols and some peculiar phenols which contribute significantly to the unique properties of EVOO, not least oxidative stability and distinguished flavour. Though oleic acid has been endorsed with multiple beneficial activities following its enrichment in the membrane lipid bilayer, as summarised in a recent review (Lopez et al. 2014), it is the minor phenolic constituents which have generated increased attention, and are now considered as predominantly responsible for most of the health-promoting properties of EVOO. Accordingly, the accumulating data on OLC and OLE reviewed in this paper collectively support the hypothesis that such phenols are capable of eliciting multiple biochemical and biological responses, that might be harnessed in counteracting several age-associated diseases and, particularly, cognitive impairment and AD (Table 1.1).

To date, the activities of EVOO phenols potentially useful against AD can be grouped into several main categories: (i) inhibition of the formation of amyloidogenic Aβ fragments from APP, (ii) promotion of Aβ peptide clearance, (iii) inhibition of Aβ and tau toxic amyloid aggregation, (iv) reduction of inflammation, (v) autophagy activation, (vi) neurogenesis stimulation and (vii) antioxidant defence activation. OLE has until now given more unequivocal positive results than OLC with regards to the inhibition of toxic amyloid aggregation and the stimulation of cellular protective mechanisms like autophagy. Moreover, the specificity and selectivity of OLC crosslinking activity still

Table 1.1 Summary of OLE and OLC activities useful in counteracting AD

	Activity	References
OLE and oleuropein	Inhibition of the formation of Aβ toxic oligomers and plaques and remodelling of pre-existing Aβ deposits *in vitro* and *in vivo*	Rigacci et al. (2011), Diomede et al. (2013), Luccarini et al. (2014), and Grossi et al. (2013)
	Improvement in survival, cognitive function and motor performance in AD transgenic models (*C. elegans*, mouse)	Diomede et al. (2013) and Grossi et al. (2013, 2014)
	Inflammation reduction	Luccarini et al. (2014), Grossi et al. (2013, 2014), Dell'Agli et al. (2010), Scoditti et al. (2012), Sindona et al. (2012), and Impellizzeri et al. (2011)
	Autophagy activation	Grossi et al. (2013, 2014)
	Stimulation of hippocampal neurogenesis and increase in dopaminergic neurons	Grossi et al. (2014) and Sarbishegi et al. (2014)
	Inhibition of tau aggregation	Daccache et al. (2011)
	Promotion of sAPPα fragment production	Kostomoiri et al. (2013)
	Antioxidant enzymes activation	Sarbishegi et al. (2014)
OLC	Reduction of Aβ oligomers binding to the membrane, protection against synaptic deterioration	Pitt et al. (2009)
	Conversion of tau monomers and oligomers into non-fibrillar aggregates	Li et al. (2009)
	Up-regulation of Aβ-degrading enzymes and Aβ transporters at the BBB	Abuznait et al. (2013)
	AMPK activation	Khanal et al. (2011)
	Inflammation reduction	Beauchamp et al. (2005) and Iacono et al. (2010)

needs to be confirmed *in vivo*, to exclude negative side effects on functional proteins. Nevertheless, the potent anti-inflammatory activity of OLC and its ability to promote Aβ degradation and clearance are valuable properties.

The molecular mechanism/s by which OLE and OLC evoke those multiple cellular responses must still be clarified. Do they enter cells and directly bind to target molecules or do they mainly interact with membranes thereby inducing a signalling cascade? Data obtained with unilamellar phosphatidylcholine vesicles using fluorescence anisotropy of probes and fluorescence quenching studies led to conclusions that both OLE and OLC remained at the surface of the lipid bilayer (Paiva-Martins et al. 2003). Yet experimental evidence provided more recently by the same authors using erythrocytes suggested that OLE not only interacts with the cellular membrane but may also penetrate into the bilayer to reach the radicals formed *in situ* (Paiva-Martins et al. 2010). One explanation for this discrepancy could be that biological membranes present microdomains of peculiar lipid composition that may facilitate phenols uptake. Accordingly, OLE (as well as oleuropein and other phenols and derivatives) was recently found inside the cytoplasm of human breast carcinoma cells exposed to an olive leaf extract (Quirantes-Pine et al. 2013) and of colon cancer cells exposed to EVOO extracts (Fernandez-Arroyo et al. 2012). These results leave the possibility of a direct intracellular signalling activity by EVOO phenols open to discussion.

Finally, in spite of the documented low bioavailability of these compounds, the positive results already obtained following *in vivo* administration confirm their efficacy, at least in model organisms. It is hoped that OLE and OLC will soon enter clinical trials to

rigorously assess their efficacy against AD during the different phases of disease progression, especially preclinical stages. Investigations employing different encapsulation strategies aimed at increasing the stability, bioavailability and brain targeting of these phenols are very promising and critical in this phase of the research.

References

Abuznait AH, Qosa H, Busnena BA, El Sayed KA, Kaddoumi A (2013) Olive-oil-derived oleocanthal enhances beta-amyloid clearance as a potential neuroprotective mechanism against Alzheimer's disease: in vitro and in vivo studies. ACS Chem Neurosci 4(6):973–982. doi:10.1021/cn400024q

Alvarez A, Toro R, Caceres A, Maccioni RB (1999) Inhibition of tau phosphorylating protein kinase cdk5 prevents beta-amyloid-induced neuronal death. FEBS Lett 459(3):421–426

Bazoti FN, Bergquist J, Markides KE, Tsarbopoulos A (2006) Noncovalent interaction between amyloid-beta-peptide (1–40) and oleuropein studied by electrospray ionization mass spectrometry. J Am Soc Mass Spectrom 17(4):568–575. doi:10.1016/j.jasms.2005.11.016

Bazoti FN, Bergquist J, Markides K, Tsarbopoulos A (2008) Localization of the noncovalent binding site between amyloid-beta-peptide and oleuropein using electrospray ionization FT-ICR mass spectrometry. J Am Soc Mass Spectrom 19(8):1078–1085. doi:10.1016/j.jasms.2008.03.011

Beauchamp GK, Keast RS, Morel D, Lin J, Pika J, Han Q, Lee CH, Smith AB, Breslin PA (2005) Phytochemistry: ibuprofen-like activity in extra-virgin olive oil. Nature 437(7055):45–46. doi:10.1038/437045a

Benaki D, Stathopoulou K, Leondiadis L, Ferderigos N, Pelecanou M, Mikros E (2009) Detection of interactions of the beta-amyloid peptide with small molecules employing transferred NOEs. J Pept Sci: Off Publ Eur Pept Soc 15(6):435–441. doi:10.1002/psc.1138

Benetou V, Trichopoulou A, Orfanos P, Naska A, Lagiou P, Boffetta P, Trichopoulos D, Greek E (2008) Conformity to traditional Mediterranean diet and cancer incidence: the Greek EPIC cohort. Br J Cancer 99(1):191–195. doi:10.1038/sj.bjc.6604418

Benilova I, Karran E, De Strooper B (2012) The toxic Abeta oligomer and Alzheimer's disease: an emperor in need of clothes. Nat Neurosci 15(3):349–357. doi:10.1038/nn.3028

Billings LM, Oddo S, Green KN, McGaugh JL, LaFerla FM (2005) Intraneuronal Abeta causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. Neuron 45(5):675–688. doi:10.1016/j.neuron.2005.01.040

Bogani P, Galli C, Villa M, Visioli F (2007) Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil. Atherosclerosis 190(1):181–186. doi:10.1016/j.atherosclerosis.2006.01.011

Brenes M, Garcia A, Garcia P, Rios JJ, Garrido A (1999) Phenolic compounds in Spanish olive oils. J Agric Food Chem 47(9):3535–3540

Brenes M, Garcia A, Garcia P, Garrido A (2000) Rapid and complete extraction of phenols from olive oil and determination by means of a coulometric electrode array system. J Agric Food Chem 48(11):5178–5183

Brenes M, Garcia A, Garcia P, Garrido A (2001) Acid hydrolysis of secoiridoid aglycons during storage of virgin olive oil. J Agric Food Chem 49(11):5609–5614

Brorsson AC, Bolognesi B, Tartaglia GG, Shammas SL, Favrin G, Watson I, Lomas DA, Chiti F, Vendruscolo M, Dobson CM, Crowther DC, Luheshi LM (2010) Intrinsic determinants of neurotoxic aggregate formation by the amyloid beta peptide. Biophys J 98(8):1677–1684. doi:10.1016/j.bpj.2009.12.4320

Cai Z, Yan LJ, Li K, Quazi SH, Zhao B (2012) Roles of AMP-activated protein kinase in Alzheimer's disease. Neruomol Med 14(1):1–14. doi:10.1007/s12017-012-8173-2

Canuelo A, Gilbert-Lopez B, Pacheco-Linan P, Martinez-Lara E, Siles E, Miranda-Vizuete A (2012) Tyrosol, a main phenol present in extra virgin olive oil, increases lifespan and stress resistance in Caenorhabditis elegans. Mech Ageing Dev 133(8):563–574. doi:10.1016/j.mad.2012.07.004

Chen CH, Zhou W, Liu S, Deng Y, Cai F, Tone M, Tone Y, Tong Y, Song W (2012) Increased NF-kappaB signalling up-regulates BACE1 expression and its therapeutic potential in Alzheimer's disease. Int J Neuropsychopharmacol/Off Sci J Coll Int Neuropsychopharmacol 15(1):77–90. doi:10.1017/S1461145711000149

Cheng KK, Yeung CF, Ho SW, Chow SF, Chow AH, Baum L (2013) Highly stabilized curcumin nanoparticles tested in an in vitro blood-brain barrier model and in Alzheimer's disease Tg2576 mice. AAPS J 15(2):324–336. doi:10.1208/s12248-012-9444-4

Chung S, Yao H, Caito S, Hwang JW, Arunachalam G, Rahman I (2010) Regulation of SIRT1 in cellular functions: role of polyphenols. Arch Biochem Biophys 501(1):79–90. doi:10.1016/j.abb.2010.05.003

Cicerale S, Conlan XA, Sinclair AJ, Keast RS (2009) Chemistry and health of olive oil phenolics. Crit Rev Food Sci Nutr 49(3):218–236. doi:10.1080/10408390701856223

Cleary JP, Walsh DM, Hofmeister JJ, Shankar GM, Kuskowski MA, Selkoe DJ, Ashe KH (2005) Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. Nat Neurosci 8(1):79–84. doi:10.1038/nn1372

Coni E, Di Benedetto R, Di Pasquale M, Masella R, Modesti D, Mattei R, Carlini EA (2000) Protective effect of oleuropein, an olive oil biophenol, on low den-

sity lipoprotein oxidizability in rabbits. Lipids 35(1): 45–54
D'Angelo S, Manna C, Migliardi V, Mazzoni O, Morrica P, Capasso G, Pontoni G, Galletti P, Zappia V (2001) Pharmacokinetics and metabolism of hydroxytyrosol, a natural antioxidant from olive oil. Drug Metab Dispos: Biol Fate Chem 29(11):1492–1498
D'Archivio M, Filesi C, Vari R, Scazzocchio B, Masella R (2010) Bioavailability of the polyphenols: status and controversies. Int J Mol Sci 11(4):1321–1342. doi:10.3390/ijms11041321
Daccache A, Lion C, Sibille N, Gerard M, Slomianny C, Lippens G, Cotelle P (2011) Oleuropein and derivatives from olives as Tau aggregation inhibitors. Neurochem Int 58(6):700–707. doi:10.1016/j.neuint.2011.02.010
de Bock M, Thorstensen EB, Derraik JG, Henderson HV, Hofman PL, Cutfield WS (2013) Human absorption and metabolism of oleuropein and hydroxytyrosol ingested as olive (Olea europaea L.) leaf extract. Mol Nutr Food Res 57(11):2079–2085. doi:10.1002/mnfr.201200795
Dell'Agli M, Fagnani R, Galli GV, Maschi O, Gilardi F, Bellosta S, Crestani M, Bosisio E, De Fabiani E, Caruso D (2010) Olive oil phenols modulate the expression of metalloproteinase 9 in THP-1 cells by acting on nuclear factor-kappaB signaling. J Agric Food Chem 58(4):2246–2252. doi:10.1021/jf9042503
Diomede L, Rigacci S, Romeo M, Stefani M, Salmona M (2013) Oleuropein aglycone protects transgenic C. elegans strains expressing Abeta42 by reducing plaque load and motor deficit. PLoS One 8(3):e58893. doi:10.1371/journal.pone.0058893
Farias G, Cornejo A, Jimenez J, Guzman L, Maccioni RB (2011) Mechanisms of tau self-aggregation and neurotoxicity. Curr Alzheimer Res 8(6):608–614
Farr SA, Price TO, Dominguez LJ, Motisi A, Saiano F, Niehoff ML, Morley JE, Banks WA, Ercal N, Barbagallo M (2012) Extra virgin olive oil improves learning and memory in SAMP8 mice. J Alzheimer's Dis: JAD 28(1):81–92. doi:10.3233/JAD-2011-110662
Feng Z, Bai L, Yan J, Li Y, Shen W, Wang Y, Wertz K, Weber P, Zhang Y, Chen Y, Liu J (2011) Mitochondrial dynamic remodeling in strenuous exercise-induced muscle and mitochondrial dysfunction: regulatory effects of hydroxytyrosol. Free Radic Biol Med 50(10):1437–1446. doi:10.1016/j.freeradbiomed.2011.03.001
Fernandez-Arroyo S, Gomez-Martinez A, Rocamora-Reverte L, Quirantes-Pine R, Segura-Carretero A, Fernandez-Gutierrez A, Ferragut JA (2012) Application of nanoLC-ESI-TOF-MS for the metabolomic analysis of phenolic compounds from extra-virgin olive oil in treated colon-cancer cells. J Pharm Biomed Anal 63:128–134. doi:10.1016/j.jpba.2012.01.033
Franconi F, Coinu R, Carta S, Urgeghe PP, Ieri F, Mulinacci N, Romani A (2006) Antioxidant effect of two virgin olive oils depends on the concentration and composition of minor polar compounds. J Agric Food Chem 54(8):3121–3125. doi:10.1021/jf053003{\mathsurround=\opskip$+$}
Galanakis PA, Bazoti FN, Bergquist J, Markides K, Spyroulias GA, Tsarbopoulos A (2011) Study of the interaction between the amyloid beta peptide (1–40) and antioxidant compounds by nuclear magnetic resonance spectroscopy. Biopolymers 96(3):316–327. doi:10.1002/bip.21558
Garcia-Villalba R, Carrasco-Pancorbo A, Nevedomskaya E, Mayboroda OA, Deelder AM, Segura-Carretero A, Fernandez-Gutierrez A (2010) Exploratory analysis of human urine by LC-ESI-TOF MS after high intake of olive oil: understanding the metabolism of polyphenols. Anal Bioanal Chem 398(1):463–475. doi:10.1007/s00216-010-3899-x
Garcia-Villalba R, Larrosa M, Possemiers S, Tomas-Barberan FA, Espin JC (2014) Bioavailability of phenolics from an oleuropein-rich olive (Olea europaea) leaf extract and its acute effect on plasma antioxidant status: comparison between pre- and postmenopausal women. Eur J Nutr 53(4):1015–1027. doi:10.1007/s00394-013-0604-9
Gilbert BJ (2013) The role of amyloid beta in the pathogenesis of Alzheimer's disease. J Clin Pathol 66(5):362–366. doi:10.1136/jclinpath-2013-201515
Granados-Principal S, Quiles JL, Ramirez-Tortosa CL, Sanchez-Rovira P, Ramirez-Tortosa MC (2010) Hydroxytyrosol: from laboratory investigations to future clinical trials. Nutr Rev 68(4):191–206. doi:10.1111/j.1753-4887.2010.00278.x
Grossi C, Rigacci S, Ambrosini S, Ed Dami T, Luccarini I, Traini C, Failli P, Berti A, Casamenti F, Stefani M (2013) The polyphenol oleuropein aglycone protects TgCRND8 mice against Ass plaque pathology. PLoS One 8(8):e71702. doi:10.1371/journal.pone.0071702
Grossi C, Ed Dami T, Rigacci S, Stefani M, Luccarini I, Casamenti F (2014) Employing Alzheimer disease animal models for translational research: focus on dietary components. Neuro-Degener Dis 13(2–3):131–134. doi:10.1159/000355461
Group AR (2006) Cardiovascular and cerebrovascular events in the randomized, controlled Alzheimer's Disease Anti-Inflammatory Prevention Trial (ADAPT). PLoS Clin Trials 1(7):e33. doi:10.1371/journal.pctr.0010033
Guzman-Martinez L, Farias GA, Maccioni RB (2013) Tau oligomers as potential targets for Alzheimer's diagnosis and novel drugs. Front Neurol 4:167. doi:10.3389/fneur.2013.00167
Hardy JA, Higgins GA (1992) Alzheimer's disease: the amyloid cascade hypothesis. Science 256(5054):184–185
Iacono A, Gomez R, Sperry J, Conde J, Bianco G, Meli R, Gomez-Reino JJ, Smith AB 3rd, Gualillo O (2010) Effect of oleocanthal and its derivatives on inflammatory response induced by lipopolysaccharide in a murine chondrocyte cell line. Arthritis Rheum 62(6):1675–1682. doi:10.1002/art.27437
Impellizzeri D, Esposito E, Mazzon E, Paterniti I, Di Paola R, Bramanti P, Morittu VM, Procopio A, Britti D, Cuzzocrea S (2011) The effects of oleuropein aglycone, an olive oil compound, in a mouse model of carrageenan-

induced pleurisy. Clin Nutr 30(4):533–540. doi:10.1016/j.clnu.2011.02.004

in t' Veld BA, Ruitenberg A, Hofman A, Launer LJ, van Duijn CM, Stijnen T, Breteler MM, Stricker BH (2001) Nonsteroidal antiinflammatory drugs and the risk of Alzheimer's disease. N Engl J Med 345(21):1515–1521. doi:10.1056/NEJMoa010178

Jacomelli M, Pitozzi V, Zaid M, Larrosa M, Tonini G, Martini A, Urbani S, Taticchi A, Servili M, Dolara P, Giovannelli L (2010) Dietary extra-virgin olive oil rich in phenolic antioxidants and the aging process: long-term effects in the rat. J Nutr Biochem 21(4):290–296. doi:10.1016/j.jnutbio.2008.12.014

Jeganathan S, von Bergen M, Mandelkow EM, Mandelkow E (2008) The natively unfolded character of tau and its aggregation to Alzheimer-like paired helical filaments. Biochemistry 47(40):10526–10539. doi:10.1021/bi800783d

Kallberg Y, Gustafsson M, Persson B, Thyberg J, Johansson J (2001) Prediction of amyloid fibril-forming proteins. J Biol Chem 276(16):12945–12950. doi:10.1074/jbc.M010402200

Khanal P, Oh WK, Yun HJ, Namgoong GM, Ahn SG, Kwon SM, Choi HK, Choi HS (2011) p-HPEA-EDA, a phenolic compound of virgin olive oil, activates AMP-activated protein kinase to inhibit carcinogenesis. Carcinogenesis 32(4):545–553. doi:10.1093/carcin/bgr001

Khymenets O, Fito M, Tourino S, Munoz-Aguayo D, Pujadas M, Torres JL, Joglar J, Farre M, Covas MI, de la Torre R (2010) Antioxidant activities of hydroxytyrosol main metabolites do not contribute to beneficial health effects after olive oil ingestion. Drug Metab Dispos: Biol Fate Chem 38(9):1417–1421. doi:10.1124/dmd.110.032821

Koffie RM, Meyer-Luehmann M, Hashimoto T, Adams KW, Mielke ML, Garcia-Alloza M, Micheva KD, Smith SJ, Kim ML, Lee VM, Hyman BT, Spires-Jones TL (2009) Oligomeric amyloid beta associates with postsynaptic densities and correlates with excitatory synapse loss near senile plaques. Proc Natl Acad Sci U S A 106(10):4012–4017. doi:10.1073/pnas.0811698106

Konstantinidou V, Covas MI, Munoz-Aguayo D, Khymenets O, de la Torre R, Saez G, Tormos Mdel C, Toledo E, Marti A, Ruiz-Gutierrez V, Ruiz Mendez MV, Fito M (2010) In vivo nutrigenomic effects of virgin olive oil polyphenols within the frame of the Mediterranean diet: a randomized controlled trial. FASEB J: Off Publ Fed Am Soc Exp Biol 24(7):2546–2557. doi:10.1096/fj.09-148452

Kostomoiri M, Fragkouli A, Sagnou M, Skaltsounis LA, Pelecanou M, Tsilibary EC, Tzinia AK (2013) Oleuropein, an anti-oxidant polyphenol constituent of olive promotes alpha-secretase cleavage of the amyloid precursor protein (AbetaPP). Cell Mol Neurobiol 33(1):147–154. doi:10.1007/s10571-012-9880-9

Krstic D, Madhusudan A, Doehner J, Vogel P, Notter T, Imhof C, Manalastas A, Hilfiker M, Pfister S, Schwerdel C, Riether C, Meyer U, Knuesel I (2012) Systemic immune challenges trigger and drive Alzheimer-like neuropathology in mice. J Neuroinflammation 9:151. doi:10.1186/1742-2094-9-151

Lasagna-Reeves CA, Castillo-Carranza DL, Jackson GR, Kayed R (2011) Tau oligomers as potential targets for immunotherapy for Alzheimer's disease and tauopathies. Curr Alzheimer Res 8(6):659–665

Lee S, Fernandez EJ, Good TA (2007) Role of aggregation conditions in structure, stability, and toxicity of intermediates in the Abeta fibril formation pathway. Prot Sci: Publ Prot Soc 16(4):723–732. doi:10.1110/ps.062514807

Li W, Sperry JB, Crowe A, Trojanowski JQ, Smith AB 3rd, Lee VM (2009) Inhibition of tau fibrillization by oleocanthal via reaction with the amino groups of tau. J Neurochem 110(4):1339–1351. doi:10.1111/j.1471-4159.2009.06224.x

Lin P, Qian W, Wang X, Cao L, Li S, Qian T (2013) The biotransformation of oleuropein in rats. Biomed Chromatogr: BMC 27(9):1162–1167. doi:10.1002/bmc.2922

Lopez S, Bermudez B, Montserrat-de la Paz S, Jaramillo S, Varela LM, Ortega-Gomez A, Abia R, Muriana FJ (2014) Membrane composition and dynamics: a target of bioactive virgin olive oil constituents. Biochim Biophys Acta 1838(6):1638–1656. doi:10.1016/j.bbamem.2014.01.007

Lopez-Miranda J, Perez-Jimenez F, Ros E, De Caterina R, Badimon L, Covas MI, Escrich E, Ordovas JM, Soriguer F, Abia R, de la Lastra CA, Battino M, Corella D, Chamorro-Quiros J, Delgado-Lista J, Giugliano D, Esposito K, Estruch R, Fernandez-Real JM, Gaforio JJ, La Vecchia C, Lairon D, Lopez-Segura F, Mata P, Menendez JA, Muriana FJ, Osada J, Panagiotakos DB, Paniagua JA, Perez-Martinez P, Perona J, Peinado MA, Pineda-Priego M, Poulsen HE, Quiles JL, Ramirez-Tortosa MC, Ruano J, Serra-Majem L, Sola R, Solanas M, Solfrizzi V, de la Torre-Fornell R, Trichopoulou A, Uceda M, Villalba-Montoro JM, Villar-Ortiz JR, Visioli F, Yiannakouris N (2010) Olive oil and health: summary of the II international conference on olive oil and health consensus report, Jaen and Cordoba (Spain) 2008. Nutr, Metab, Cardiovasc Dis: NMCD 20(4):284–294. doi:10.1016/j.numecd.2009.12.007

Luccarini I, Ed Dami T, Grossi C, Rigacci S, Stefani M, Casamenti F (2014) Oleuropein aglycone counteracts Abeta42 toxicity in the rat brain. Neurosci Lett 558:67–72. doi:10.1016/j.neulet.2013.10.062

Luo C, Li Y, Wang H, Cui Y, Feng Z, Li H, Li Y, Wang Y, Wurtz K, Weber P, Long J, Liu J (2013) Hydroxytyrosol promotes superoxide production and defects in autophagy leading to anti-proliferation and apoptosis on human prostate cancer cells. Curr Cancer Drug Targets 13(6):625–639

Lyman M, Lloyd DG, Ji X, Vizcaychipi MP, Ma D (2014) Neuroinflammation: the role and consequences. Neurosci Res 79:1–12. doi:10.1016/j.neures.2013.10.004

Maccioni RB, Munoz JP, Barbeito L (2001) The molecular bases of Alzheimer's disease and other neurodegenerative disorders. Arch Med Res 32(5):367–381

Markopoulos C, Vertzoni M, Agalias A, Magiatis P, Reppas C (2009) Stability of oleuropein in the human proximal gut. J Pharm Pharmacol 61(2):143–149. doi:10.1211/jpp/61.02.0002

Martinez-Gonzalez MA, Garcia-Lopez M, Bes-Rastrollo M, Toledo E, Martinez-Lapiscina EH, Delgado-Rodriguez M, Vazquez Z, Benito S, Beunza JJ (2011) Mediterranean diet and the incidence of cardiovascular disease: a Spanish cohort. Nutr, Metab, Cardiovas Dis: NMCD 21(4):237–244. doi:10.1016/j.numecd.2009.10.005

Martinez-Lapiscina EH, Clavero P, Toledo E, San Julian B, Sanchez-Tainta A, Corella D, Lamuela-Raventos RM, Martinez JA, Martinez-Gonzalez MA (2013) Virgin olive oil supplementation and long-term cognition: the PREDIMED-NAVARRA randomized, trial. J Nutr, Health Aging 17(6):544–552. doi:10.1007/s12603-013-0027-6

McGeer PL, McGeer EG (2013) The amyloid cascade-inflammatory hypothesis of Alzheimer disease: implications for therapy. Acta Neuropathol 126(4):479–497. doi:10.1007/s00401-013-1177-7

Meinhardt J, Sachse C, Hortschansky P, Grigorieff N, Fandrich M (2009) Abeta(1–40) fibril polymorphism implies diverse interaction patterns in amyloid fibrils. J Mol Biol 386(3):869–877. doi:10.1016/j.jmb.2008.11.005

Menendez JA, Joven J, Aragones G, Barrajon-Catalan E, Beltran-Debon R, Borras-Linares I, Camps J, Corominas-Faja B, Cufi S, Fernandez-Arroyo S, Garcia-Heredia A, Hernandez-Aguilera A, Herranz-Lopez M, Jimenez-Sanchez C, Lopez-Bonet E, Lozano-Sanchez J, Luciano-Mateo F, Martin-Castillo B, Martin-Paredero V, Perez-Sanchez A, Oliveras-Ferraros C, Riera-Borrull M, Rodriguez-Gallego E, Quirantes-Pine R, Rull A, Tomas-Menor L, Vazquez-Martin A, Alonso-Villaverde C, Micol V, Segura-Carretero A (2013) Xenohormetic and anti-aging activity of secoiridoid polyphenols present in extra virgin olive oil: a new family of gerosuppressant agents. Cell Cycle 12(4):555–578. doi:10.4161/cc.23756

Meraz-Rios MA, Toral-Rios D, Franco-Bocanegra D, Villeda-Hernandez J, Campos-Pena V (2013) Inflammatory process in Alzheimer's disease. Front Integr Neurosci 7:59. doi:10.3389/fnint.2013.00059

Miro-Casas E, Covas MI, Farre M, Fito M, Ortuno J, Weinbrenner T, Roset P, de la Torre R (2003) Hydroxytyrosol disposition in humans. Clin Chem 49(6 Pt 1):945–952

Mohamed A, Cortez L, de Chaves EP (2011) Aggregation state and neurotoxic properties of alzheimer beta-amyloid peptide. Curr Protein Pept Sci 12(3):235–257

Monti MC, Margarucci L, Tosco A, Riccio R, Casapullo A (2011) New insights on the interaction mechanism between tau protein and oleocanthal, an extra-virgin olive-oil bioactive component. Food Funct 2(7):423–428. doi:10.1039/c1fo10064e

Mourtzinos I, Salta F, Yannakopoulou K, Chiou A, Karathanos VT (2007) Encapsulation of olive leaf extract in beta-cyclodextrin. J Agric Food Chem 55(20):8088–8094. doi:10.1021/jf0709698

Paiva-Martins F, Gordon MH, Gameiro P (2003) Activity and location of olive oil phenolic antioxidants in liposomes. Chem Phys Lipids 124(1):23–36

Paiva-Martins F, Fernandes J, Santos V, Silva L, Borges F, Rocha S, Belo L, Santos-Silva A (2010) Powerful protective role of 3,4-dihydroxyphenylethanol-elenolic acid dialdehyde against erythrocyte oxidative-induced hemolysis. J Agric Food Chem 58(1):135–140. doi:10.1021/jf9031052

Pan XD, Zhu YG, Lin N, Zhang J, Ye QY, Huang HP, Chen XC (2011) Microglial phagocytosis induced by fibrillar beta-amyloid is attenuated by oligomeric beta-amyloid: implications for Alzheimer's disease. Mol Neurodegener 6:45. doi:10.1186/1750-1326-6-45

Pierno S, Tricarico D, Liantonio A, Mele A, Digennaro C, Rolland JF, Bianco G, Villanova L, Merendino A, Camerino GM, De Luca A, Desaphy JF, Camerino DC (2014) An olive oil-derived antioxidant mixture ameliorates the age-related decline of skeletal muscle function. AGE 36(1):73–88. doi:10.1007/s11357-013-9544-9

Pitozzi V, Jacomelli M, Catelan D, Servili M, Taticchi A, Biggeri A, Dolara P, Giovannelli L (2012) Long-term dietary extra-virgin olive oil rich in polyphenols reverses age-related dysfunctions in motor coordination and contextual memory in mice: role of oxidative stress. Rejuvenation Res 15(6):601–612. doi:10.1089/rej.2012.1346

Pitt J, Roth W, Lacor P, Smith AB 3rd, Blankenship M, Velasco P, De Felice F, Breslin P, Klein WL (2009) Alzheimer's-associated Abeta oligomers show altered structure, immunoreactivity and synaptotoxicity with low doses of oleocanthal. Toxicol Appl Pharmacol 240(2):189–197. doi:10.1016/j.taap.2009.07.018

Quirantes-Pine R, Zurek G, Barrajon-Catalan E, Bassmann C, Micol V, Segura-Carretero A, Fernandez-Gutierrez A (2013) A metabolite-profiling approach to assess the uptake and metabolism of phenolic compounds from olive leaves in SKBR3 cells by HPLC-ESI-QTOF-MS. J Pharm Biomed Anal 72:121–126. doi:10.1016/j.jpba.2012.09.029

Rafehi H, Smith AJ, Balcerczyk A, Ziemann M, Ooi J, Loveridge SJ, Baker EK, El-Osta A, Karagiannis TC (2012) Investigation into the biological properties of the olive polyphenol, hydroxytyrosol: mechanistic insights by genome-wide mRNA-Seq analysis. Gene Nutr 7(2):343–355. doi:10.1007/s12263-011-0249-3

Rescifina A, Chiacchio U, Iannazzo D, Piperno A, Romeo G (2010) beta-cyclodextrin and caffeine complexes with natural polyphenols from olive and olive oils: NMR, thermodynamic, and molecular modeling studies. J Agric Food Chem 58(22):11876–11882. doi:10.1021/jf1028366

Rigacci S, Berti A (2009) Oleuropein aglycon: the bitter treasure of extra virgin olive oil. In: Corrigan JD (ed) Olive oil and health. Nova, New York, pp 221–240

Rigacci S, Guidotti V, Bucciantini M, Parri M, Nediani C, Cerbai E, Stefani M, Berti A (2010) Oleuropein aglycon prevents cytotoxic amyloid aggregation of human amylin. J Nutr Biochem 21(8):726–735. doi:10.1016/j.jnutbio.2009.04.010

Rigacci S, Guidotti V, Bucciantini M, Nichino D, Relini A, Berti A, Stefani M (2011) Abeta(1–42) aggregates into non-toxic amyloid assemblies in the presence of the natural polyphenol oleuropein aglycon. Curr Alzheimer Res 8(8):841–852

Romani A, Mulinacci N, Pinelli P, Vincieri FF, Cimato A (1999) Polyphenolic content in five tuscany cultivars of Olea europaea L. J Agric Food Chem 47(3):964–967

Romero C, Medina E, Vargas J, Brenes M, De Castro A (2007) In vitro activity of olive oil polyphenols against Helicobacter pylori. J Agric Food Chem 55(3):680–686. doi:10.1021/jf0630217

Sa F, Pinto P, Cunha C, Lemos R, Letra L, Simoes M, Santana I (2012) Differences between early and late-onset Alzheimer's disease in neuropsychological tests. Front Neurol 3:81. doi:10.3389/fneur.2012.00081

Sarbishegi M, Mehraein F, Soleimani M (2014) Antioxidant role of oleuropein on midbrain and dopaminergic neurons of substantia nigra in aged rats. Iran Biomed J 18(1):16–22

Sarsour EH, Kumar MG, Kalen AL, Goswami M, Buettner GR, Goswami PC (2012) MnSOD activity regulates hydroxytyrosol-induced extension of chronological lifespan. AGE 34(1):95–109. doi:10.1007/s11357-011-9223-7

Scharf S, Mander A, Ugoni A, Vajda F, Christophidis N (1999) A double-blind, placebo-controlled trial of diclofenac/misoprostol in Alzheimer's disease. Neurology 53(1):197–201

Scoditti E, Calabriso N, Massaro M, Pellegrino M, Storelli C, Martines G, De Caterina R, Carluccio MA (2012) Mediterranean diet polyphenols reduce inflammatory angiogenesis through MMP-9 and COX-2 inhibition in human vascular endothelial cells: a potentially protective mechanism in atherosclerotic vascular disease and cancer. Arch Biochem Biophys 527(2):81–89. doi:10.1016/j.abb.2012.05.003

Serpell LC, Sunde M, Benson MD, Tennent GA, Pepys MB, Fraser PE (2000) The protofilament substructure of amyloid fibrils. J Mol Biol 300(5):1033–1039. doi:10.1006/jmbi.2000.3908

Serra A, Rubio L, Borras X, Macia A, Romero MP, Motilva MJ (2012) Distribution of olive oil phenolic compounds in rat tissues after administration of a phenolic extract from olive cake. Mol Nutr Food Res 56(3):486–496. doi:10.1002/mnfr.201100436

Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT (2011) Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med 1(1):a006189. doi:10.1101/cshperspect.a006189

Servili M, Baldioli M, Selvaggini R, Macchioni A, Montedoro G (1999) Phenolic compounds of olive fruit: one- and two-dimensional nuclear magnetic resonance characterization of Nuzhenide and its distribution in the constitutive parts of fruit. J Agric Food Chem 47(1):12–18

Servili M, Esposto S, Fabiani R, Urbani S, Taticchi A, Mariucci F, Selvaggini R, Montedoro GF (2009) Phenolic compounds in olive oil: antioxidant, health and organoleptic activities according to their chemical structure. Inflammopharmacology 17(2):76–84. doi:10.1007/s10787-008-8014-y

Silverberg GD, Messier AA, Miller MC, Machan JT, Majmudar SS, Stopa EG, Donahue JE, Johanson CE (2010) Amyloid efflux transporter expression at the blood-brain barrier declines in normal aging. J Neuropathol Exp Neurol 69(10):1034–1043. doi:10.1097/NEN.0b013e3181f46e25

Sindona G, Caruso A, Cozza A, Fiorentini S, Lorusso B, Marini E, Nardi M, Procopio A, Zicari S (2012) Anti-inflammatory effect of 3,4-DHPEA-EDA [2-(3,4 -hydroxyphenyl) ethyl (3S, 4E)-4-formyl-3-(2-oxoethyl)hex-4-enoate] on primary human vascular endothelial cells. Curr Med Chem 19(23):4006–4013

Sofi F, Abbate R, Gensini GF, Casini A (2010) Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. Am J Clin Nutr 92(5):1189–1196. doi:10.3945/ajcn.2010.29673

Stefani M (2012) Structural features and cytotoxicity of amyloid oligomers: implications in Alzheimer's disease and other diseases with amyloid deposits. Prog Neurobiol 99(3):226–245. doi:10.1016/j.pneurobio.2012.03.002

St-Laurent-Thibault C, Arseneault M, Longpre F, Ramassamy C (2011) Tyrosol and hydroxytyrosol, two main components of olive oil, protect N2a cells against amyloid-beta-induced toxicity. Involvement of the NF-kappaB signaling. Curr Alzheimer Res 8(5):543–551

Suarez M, Romero MP, Motilva MJ (2010) Development of a phenol-enriched olive oil with phenolic compounds from olive cake. J Agric Food Chem 58(19):10396–10403. doi:10.1021/jf102203x

Tasset I, Pontes AJ, Hinojosa AJ, de la Torre R, Tunez I (2011) Olive oil reduces oxidative damage in a 3-nitropropionic acid-induced Huntington's disease-like rat model. Nutr Neurosci 14(3):106–111. doi:10.1179/1476830511Y.0000000005

Tjernberg LO, Naslund J, Lindqvist F, Johansson J, Karlstrom AR, Thyberg J, Terenius L, Nordstedt C (1996) Arrest of beta-amyloid fibril formation by a pentapeptide ligand. J Biol Chem 271(15):8545–8548

Tjernberg LO, Callaway DJ, Tjernberg A, Hahne S, Lilliehook C, Terenius L, Thyberg J, Nordstedt C (1999) A molecular model of Alzheimer amyloid beta-peptide fibril formation. J Biol Chem 274(18):12619–12625

Tovar MJ, Motilva MJ, Romero MP (2001) Changes in the phenolic composition of virgin olive oil from young trees (Olea europaea L. cv. Arbequina) grown under linear irrigation strategies. J Agric Food Chem 49(11):5502–5508

Tripoli E, Giammanco M, Tabacchi G, Di Majo D, Giammanco S, La Guardia M (2005) The phenolic compounds of olive oil: structure, biological activity

and beneficial effects on human health. Nutr Res Rev 18(1):98–112. doi:10.1079/NRR200495

Valls-Pedret C, Lamuela-Raventos RM, Medina-Remon A, Quintana M, Corella D, Pinto X, Martinez-Gonzalez MA, Estruch R, Ros E (2012) Polyphenol-rich foods in the Mediterranean diet are associated with better cognitive function in elderly subjects at high cardiovascular risk. J Alzheimer's Dis: JAD 29(4):773–782. doi:10.3233/JAD-2012-111799

Vissers MN, Zock PL, Roodenburg AJ, Leenen R, Katan MB (2002) Olive oil phenols are absorbed in humans. J Nutr 132(3):409–417

Vlad SC, Miller DR, Kowall NW, Felson DT (2008) Protective effects of NSAIDs on the development of Alzheimer disease. Neurology 70(19):1672–1677. doi:10.1212/01.wnl.0000311269.57716.63

Vogelgesang S, Warzok RW, Cascorbi I, Kunert-Keil C, Schroeder E, Kroemer HK, Siegmund W, Walker LC, Pahnke J (2004) The role of P-glycoprotein in cerebral amyloid angiopathy; implications for the early pathogenesis of Alzheimer's disease. Curr Alzheimer Res 1(2):121–125

Walsh DM, Selkoe DJ (2004) Oligomers on the brain: the emerging role of soluble protein aggregates in neurodegeneration. Prot Pept Lett 11(3): 213–228

Walsh DM, Hartley DM, Kusumoto Y, Fezoui Y, Condron MM, Lomakin A, Benedek GB, Selkoe DJ, Teplow DB (1999) Amyloid beta-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. J Biol Chem 274(36):25945–25952

Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ (2002) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature 416(6880):535–539. doi:10.1038/416535a

Weinbrenner T, Fito M, de la Torre R, Saez GT, Rijken P, Tormos C, Coolen S, Albaladejo MF, Abanades S, Schroder H, Marrugat J, Covas MI (2004) Olive oils high in phenolic compounds modulate oxidative/antioxidative status in men. J Nutr 134(9):2314–2321

Zrelli H, Matsuoka M, Kitazaki S, Zarrouk M, Miyazaki H (2011) Hydroxytyrosol reduces intracellular reactive oxygen species levels in vascular endothelial cells by upregulating catalase expression through the AMPK-FOXO3a pathway. Eur J Pharmacol 660(2–3):275–282. doi:10.1016/j.ejphar.2011.03.045

Alzheimer's Disease, *Drosophila melanogaster* and Polyphenols

2

Marlene Jimenez-Del-Rio and Carlos Velez-Pardo

Abstract

Alzheimer's disease (AD) is an insidious neurological disorder that affects memory, one of the human brain's main cognitive functions. Around 5.2 million Americans currently have AD, and the number threatens to climb to 7 million by 2020. Our native country, Colombia, is no exception with an estimated 260,000 individuals to be affected by AD in 2020. A large, genetically-isolated community in Antioquia, Colombia, with early-onset familial Alzheimer's disease due to a presenilin-1 mutation is ideally suited for the study of molecular mechanisms of AD, and hence accelerate the discovery of new or alternative treatment approaches. In this regard, polyphenols – also known as polyhydroxyphenols – have shown antioxidant activity, gene regulation, metal chelator and anti-amyloidogenic aggregation effects. However, further *in vitro* and *in vivo* investigations are warranted to validate their use in clinical trials. *Drosophila melanogaster* is increasingly being used as a valid *in vivo* model of AD. Here, we summarise data published within the past 16 years (1998–2014) on the molecular biology of AD and the use of polyphenols in the fly to understand the molecular actions and feasibility of these compounds in the treatment of AD.

Keywords

Alzheimer's disease • Amyloid-beta • *Drosophila melanogaster* • Lymphocytes • Oxidative stress • Polyphenols

2.1 Global Alzheimer's Madness: Is This the End of the Road?

In 1906, Dr. Alois Alzheimer reported the clinical symptoms and neuropathology findings of a "peculiar disease" from his patient Augusta

M. Jimenez-Del-Rio (✉) • C. Velez-Pardo (✉)
Neuroscience Research Group, Medical Research Institute, Faculty of Medicine, University of Antioquia (UdeA), SIU, Calle 70 No. 52-21, and Calle 62 # 52-59, Building 1, Room 412, Medellin, Colombia
e-mail: marlene.jimenez@udea.edu.co; calberto.velez@udea.edu.co

N. Vassallo (ed.), *Natural Compounds as Therapeutic Agents for Amyloidogenic Diseases*, Advances in Experimental Medicine and Biology 863, DOI 10.1007/978-3-319-18365-7_2

Deter (Stelzmann et al. 1995). The patient, a woman of 51 years, was completely disoriented in time and space and was incapable of understanding anything or of recognizing her surroundings. Most importantly, she lost her capacity to memorize. At autopsy, the brain was atrophic. After histological examination with Bielschowsky's silver stain, Alzheimer observed fibrils aggregated into dense bundles and miliary foci, which were caused by deposition of an unusual "substance" in the cerebral cortex (Alzheimer (Munich): About a peculiar disease of the cerebral cortex 2000). Although his report ignited no discussion at the time, it has fascinated the medical and research community for more than 100 years. Alzheimer's patient not only presented cognitive and language deficits but also suffered from non-cognitive features such as hallucinations, delusions, paranoia and aggressive behavior, typical of psychiatric symptoms; and presented two prominent post-mortem brain features: the dense bundles of fibrils and deposits in the cerebral cortex, later recognized as neurofibrillary tangles (NFT) and senile plaques (SP), respectively (Graeber et al. 1998). These features are the hallmarks of what is presently known as Alzheimer's disease (AD, OMIM entry 104300), and have changed little since then (Esiri 2001). Further biochemical studies have shown that NFT represent the intracellular accumulation of hyperphosphorylated tau (τ) protein (Iqbal et al. 1993; Grundke-Iqbal et al. 1986), and SP are extracellular deposits due to aggregation of the hydrophobic amyloid-β (Aβ) peptide (Glenner and Wong 1984; Masters et al. 1985). The disorder was named after Alzheimer by Emil Krapelin in 1910 (Schottyky 1932), not without controversy however (Cipriani et al. 2011; Ramirez-Bermudez 2012). Alzheimer's disease has become the most frequent neurodegenerative disease worldwide. Indeed, an estimated 5.2 million Americans have AD (Fargo and Bleiler 2014) with estimated healthcare costs spiraling to $172 billion per year (Thies et al. 2013). By 2050, the total number of people with AD dementia is projected to be 13.8 million, with 7.0 million aged 85 years or older (Hebert et al. 2013). A similar trend is observed all over the world (Chan et al. 2013). It was estimated that 35.6 million people lived with dementia worldwide in 2010, with numbers expected to almost double every 20 years, to 65.7 million in 2030 and 115.4 million in 2050 (Prince et al. 2013). Colombia is no exception. By 2020, it is estimated that about 260,000 and 1,500 individuals will be affected by sporadic and familial AD in Colombia, respectively (Unicesi 2013).

Most disturbing, however, is the fact that despite many decades of intensive research no effective and/or definitive therapeutic treatment aimed at reducing or retarding the clinic and pathologic symptoms induced by this devastating neurologic disorder is at present available. These still unmet needs call for ever-more concerted and focused efforts in basic, clinic and applied research to save human lives from a death without *memoir* (Weuve et al. 2014). Since direct study, *in vivo*, of the human brain is either technically challenging or at best extremely limited, the biochemical mechanisms involved in neuronal loss have mainly been studied in biological models.

The fly *Drosophila melanogaster* has been used as valid *in vivo* model of AD. For the scope of this review, PubMed and Scopus searches were conducted to find data within the past 16 years (1998–2014) involving the molecular biology of AD and the use of polyphenols in the fly to understand the molecular actions and feasibility of these compounds in the treatment of AD. Additionally, the authors propose a cell-death molecular mechanism induced by oxidative stress (OS) as a minimal working *in vitro* and *in vivo* model of AD to better understand neuronal demise and to effectively design antioxidant therapeutic strategies against AD.

2.2 Alzheimer Is Knocking on Paisas' Doors

In 1991, Goate and colleagues discovered the first mutation (Val717Ile) linked to an inherited form of AD, in the amyloid precursor protein (AβPP) gene on chromosome 21q21.2. The mutation was found to be linked to inherited cases of early-onset AD (EOAD) (Goate et al. 1991). By 1995,

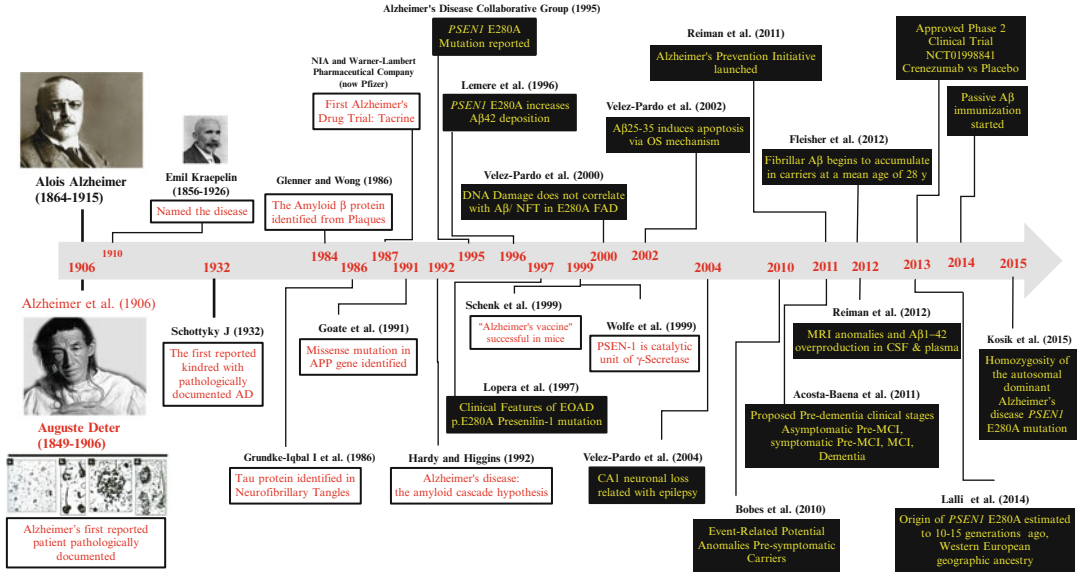


Fig. 2.1 *More than 100 years of solitude*. Historical timeline of important discoveries in Alzheimer's disease and the presenilin 1 *E280A* (g.50024 A > C, c.839A > C, p.E280A) autosomal-dominant familial mutant

Sherrington and co-workers (Sherrington et al. 1995) reported for the first time 5 mutations in the presenilin 1 (*PSEN 1*) gene located on chromosome 14q24.3 which were associated with EOAD. That same year, the Alzheimer´s Disease Collaborative Group reported the structure of *PSEN 1* and identified the mutation p.E280A, a glutamic acid-to-alanine mutation at codon 280 (g.50024 A > C, c.839A > C, Ex 8) (Alzheimer's Disease Collaborative 1995). Similar to the APP mutation, the *PSEN 1* p.E280A mutation causes EOAD. This finding clarified previous descriptions of a family affected by Alzheimer-type presenile dementia with familial segregation in Antioquia, Colombia (Cornejo et al. 1987; Lopera et al. 1994). These initial observations in turn paved the way for important genetic, molecular and cellular, clinical, cognitive and neuropathological characterization of the *PSEN 1* mutation in the Antioquian kindred, also known as the "Paisas" people (Lopera et al. 1997; Velez-Pardo et al. 2004; for a review see Sepulveda-Falla et al. 2012). The "Paisas" are a genetically isolated community with more than 5,000 individuals (Arcos-Burgos and Muenke 2002). It is worth mentioning that the age and geographic origin of c.839A > C mutation are consistent with a single founder (i.e. a *de novo* mutation event 15 generations ago) dating from the time of the Spanish Conquistadors who began colonizing Colombia during the early sixteenth century (Lalli et al. 2014). Interestingly, the search for a *PSEN 1* mutation in DNA extracted from a histological section of Auguste Deter's brain showed a T > C substitution at position 526 (c.526 T > C) resulting in a phenylalanine-to-leucine aminoacid changed at codon 176 (p.F176L) (Müller et al. 2013). This finding, a mutation in *PSEN 1* as in the Colombian study, revealed the cause of the disease and explained the histopathological observations previously noted by Alzheimer in this notable historical case. The most important findings associated with the *PSEN 1* p.E280A mutation and AD are outlined in Fig. 2.1.

By 2003, it was clear that PSEN 1 (an aspartyl protease at its catalytic core (Wolfe et al. 1999; for a review, see De Strooper et al. 2012)) together with nicastrin (Nct), anterior pharynx defective 1 (Aph-1) and presenilin enhancer 2 (Pen 2) generate an active γ-secretase complex (Kimberly et al. 2003b; De Strooper 2003). This multimolecular complex (Haass 2004)

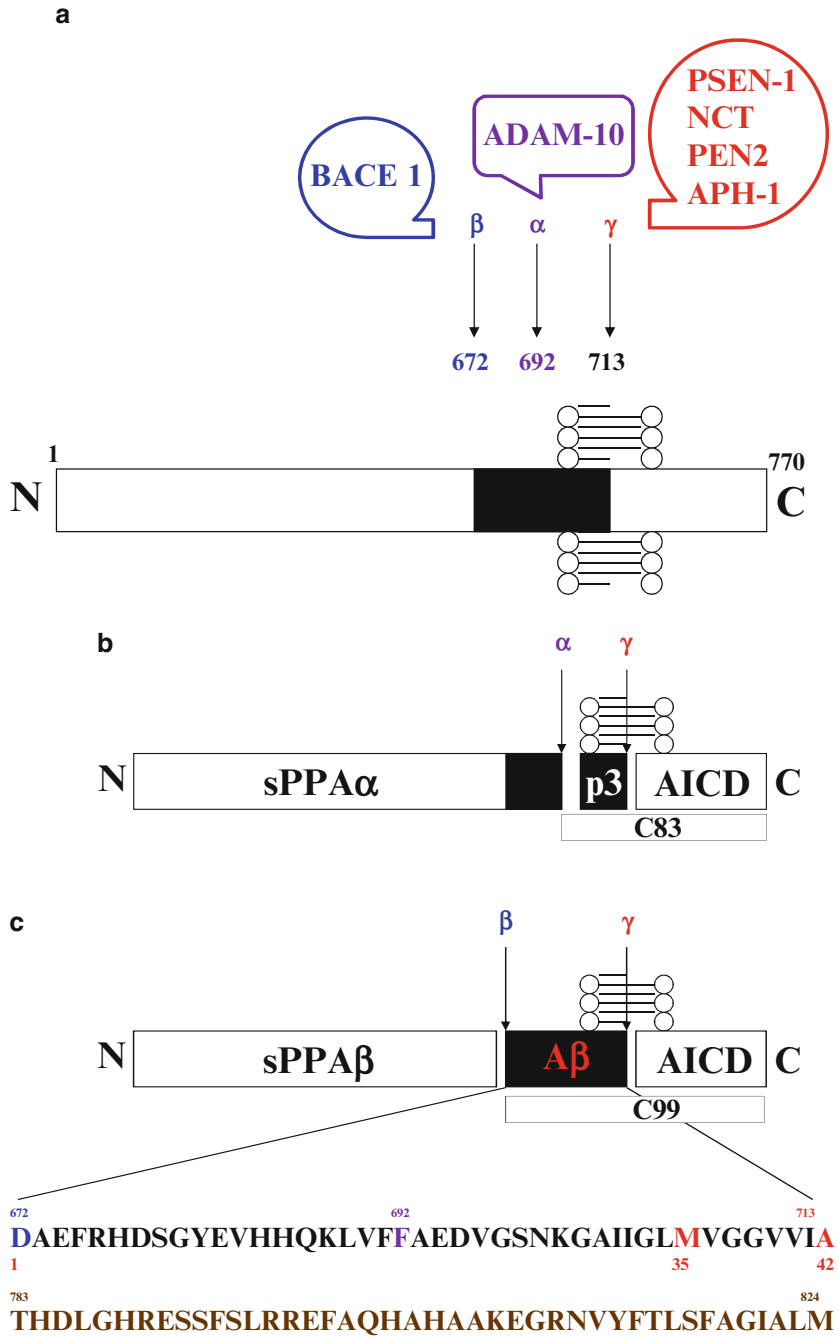


Fig. 2.2 Amyloid-β precursor protein (APP) metabolism. The APP is initially cleaved at either the α- (692 aa) or β-(672 aa) site (**a**) by α-secretase (i.e. ADAM-10) and β-secretase (i.e. BACE-1) to release the large ectodomains, designated APPsα (**b**) and APPsβ (**c**), respectively. The ectodomain shedding leaves a membrane-embedded fragment, C83 or C99, which is a substrate for γ-secretase complex cleavage (i.e. PSEN-1, PEN-2, NCT, APH-1 complex). Proteolysis by γ-secretase complex releases the p3 (**b**) and Aβ fragments (from 673 to 713 aa) at γ-site cleavage (**c**) as well as the carbonyl terminal-γ (ε-site cleavage), which forms the AICD. Protein aminoacid alignment between *Homo sapiens* Aβ(1–42) (in red) and *Drosophila melanogaster* (in brown) shows neither similarity nor identity. (**d**) The lonely pair electrons of methionine-35 residue in Aβ can react with oxygen to form both a sulfuranyl cation radical ($-S\bullet^{+}$) and anion superoxide radical ($O_2\bullet$), which can then either initiate the process of lipid peroxidation or H_2O_2 generation, respectively

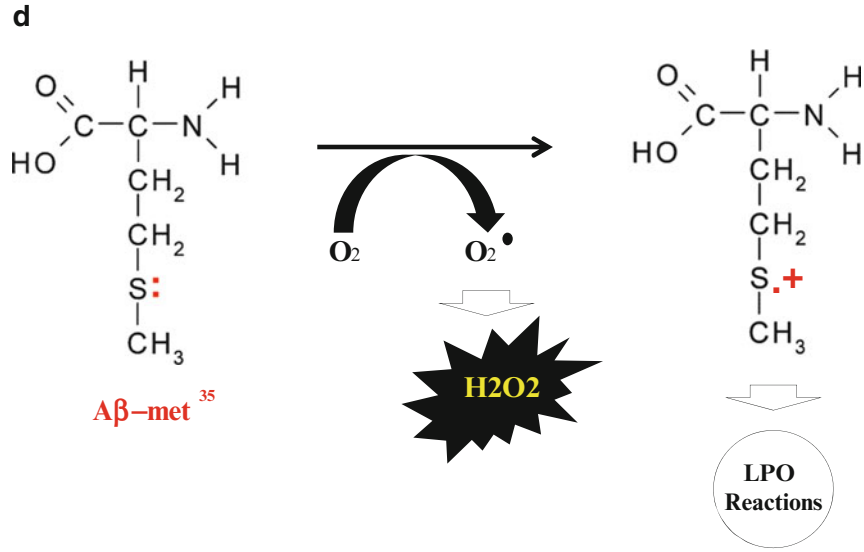


Fig. 2.2 (continued)

cleaves many type-I transmembrane proteins including AβPP (De Strooper et al. 1998) and Notch (Kimberly et al. 2003a) (Fig. 2.2a). It turns out that the AβPP is first cleaved by two other secretases – the α-secretase (also known as ADAM-10, for a-disintegrin and metalloprotease, (Kuhn et al. 2010)) or β-secretase (commonly known as BACE 1, for beta-site APP-cleaving enzyme, (Vassar et al. 1999)) to form the C83 (Fig. 2.2b) and C99 (Fig. 2.2c) residue membrane-bound fragments, respectively. C83 and C99 are substrates for a second cleavage (at the so-called γ-site) by the γ-secretase activity to form the p3 (non-amyloidogenic) and amyloid-β (Aβ) peptides, mainly Aβ(1–40) and Aβ(1–42), the latter being amyloidogenic (Fig. 2.2c). Further γ-secretase cleavage along the transmembrane domain at the so-called ε-site (A^{42}TVIVITL^{49}) generates the APP intracellular domain (AICD) (Fig. 2.2c). The function of AICD is unknown (Beckett et al. 2012). At present, several mutations in *PSEN1* (including the c.839A > C), *PSEN 2* (a homologue gene localized on chromosome 1q42.13) and *APP* gene (www.molgen.vib-ua.be/ADMutations/) have been demonstrated to modulate the position of this ε-site and to increase the ratio of the 42-residue form of Aβ versus the 40-residue Aβ (Aβ42:Aβ40), thus defining a common AD phenotype caused by those mutations (Sato et al. 2003). Despite this advanced knowledge, exactly why the γ-secretase reaction results in more Aβ(1–42) and less Aβ(1–40) is not yet clear.

Nevertheless, these exciting developments have provided a biochemical framework for the emergence of the "amyloid cascade hypothesis" (Hardy and Higgins 1992). The hypothesis posits that deposition of the Aβ peptide in the brain parenchyma initiates a sequence of events that lead to the formation of paired helical filaments (PHFs) of tau aggregates and, ultimately, result in NFT formation, neuronal loss and AD dementia. This hypothesis has been modified and/or re-examined over the years (e.g. Hardy and Selkoe 2002; Golde et al. 2006; Hardy 2006, 2009; Korczyn 2008; Karran et al. 2011; Golde et al. 2011; Giacobini and Gold 2013; Drachman 2014). The amyloid cascade hypothesis, as it currently stands, suggests that synaptotoxicity and neurotoxicity may be mediated by transient Aβ peptide forms lying between free, soluble Aβ monomers and/or insoluble amyloid fibrils (Nimmrich and Ebert 2009; Mucke and Selkoe 2012; Hubin et al. 2014). The intermediary Aβ forms most probably damage neuronal synapsis either via Aβ-receptor interactions (e.g. leukocyte

immunoglobulin-like receptor B2, LilrB2 (Kim et al. 2013)) or by Aβ pore-membrane formation (Parodi et al. 2010; Sepúlveda et al. 2014).

Such observations imply that Aβ might lead to early brain atrophy in familial Alzheimer disease (FAD) mutation carriers (Cash et al. 2013). Recent clinical studies from the Colombian "Paisas" families bearing the *PSEN 1* E280A mutation support this view. Indeed, Acosta-Baena et al. (2011) have proposed that clinical deterioration in p.E280A carriers can be detected as measurable cognitive impairment around two decades before dementia onset. Remarkably, the median age at onset was 35 years for asymptomatic pre-mild cognitive impairment (MCI), 38 years (37–40) for symptomatic pre-MCI, 44 years (43–45) for MCI, and 49 years (49–50) for dementia. It was also calculated that the median time of progression from asymptomatic to symptomatic pre-MCI was just 4 years (95 % CI 2–8). This data was further supported when it was found that fibrillar Aβ began to accumulate in *PSEN 1* mutation carriers at a mean age of 28 years, about 16 years and 21 years before the predicted median ages at MCI and dementia onset, respectively (Fleisher et al. 2012). Furthermore, Reiman and co-workers reported that, although the carrier and non-carrier groups did not differ significantly in their dementia ratings, neuropsychological test scores, or proportion of apolipoprotein E (*APOE*) ε4 carriers, the *PSEN 1* carriers had greater right hippocampal and parahippocampal activation, less precuneus and posterior cingulate deactivation, and less gray matter in several parietal regions (Reiman et al. 2012). Further Aβ(1–42) quantification in cerebrospinal fluid (CSF) and plasma showed that carriers had higher CSF concentrations and plasma Aβ(1–42) concentrations than non-carriers. These data clearly showed that young adults at genetic risk for autosomal dominant Alzheimer's disease have functional and structural MRI findings, as well as CSF and plasma biomarker findings, consistent with Aβ(1–42) overproduction. Assessment of specific brain metabolites levels by two-dimensional proton magnetic resonance spectroscopy (^{1}H-MRS) in the posterior cingulate gyrus and precuneus are potentially sensitive and specific noninvasive biomarkers of subclinical emergence of AD caused by the *PSEN1* p.E280A mutation (Londono et al. 2013). Further, cognitively normal carriers of the p.E280A mutation have a thinner cerebral cortex in angular gyrus, precuneus and superior parietal lobule regions with trend-level effects in the medial temporal lobe compared to non-carriers, as measured by T1-weighted volumetric MRI (Quiroz et al. 2013; Bobes et al. 2010). Collectively, these data suggest that early overproduction of Aβ by *PSEN 1* E280A (Lemere et al. 1996) induces a pre-clinical stage in individuals genetically determined to develop AD (Acosta-Baena et al. 2011) that is detectable either by noninvasive techniques that evaluate the brain's chemistry/structure *in vivo* such as by ^{1}H-MRS and volumetric/functional MRI imaging, or by biochemical measurements of Aβ(1–42) in CSF/plasma.

These observations imply that Aβ-induced neurodegeneration precedes aggregation in presymptomatic *PSEN 1* mutation carriers, and that the neuronal injury might be the result of either free, soluble Aβ species and/or insoluble amyloid aggregates not detectable at very early stages of the disease (Burgold et al. 2014). Furthermore, it is anticipated that presymptomatic, pre-MCI *PSEN 1* mutant carriers might develop a "pre-plaque-only" or "plaque-only" form of AD similar to the histopathology findings in the brain of Johann F., Alzheimer's second patient, who lacked NFT (Klünemann et al. 2002). This clearly implies that Aβ might cause neuronal dysfunction at an early stage of AD independently of tau protein pathology (Palop and Mucke 2010). We propose that complete corroboration of these assumptions might come from functional, structural and biochemical brain studies in adolescents, or even children (6–10 years old) that are carriers of the *PSEN 1* c.839A > C mutation (Dean et al. 2014).

How Aβ induces memory loss and neuronal death is still unknown. However, at least three different mechanisms have been proposed to explain the Aβ-induced neurotoxicity: an extracellular mechanism (i.e. the amyloid cascade hypothesis), an intracellular mechanism (Capetillo-Zarate et al. 2012; Takahashi et al. 2013) and

Aβ-induced oxidative stress (Butterfield et al. 2013). Elucidating which mechanism is truly operative, or which is hierarchically relevant *in vivo* is essential for therapeutic success (Golde 2009). To further complicate matters, the co-occurrence of Alzheimer's Aβ and tau at synaptic terminals has been demonstrated (Takahashi et al. 2010). This observation implies tau as a mediator for Alzheimer's disease-related synaptic deficits (Liao et al. 2014). Therefore, AD is a genetically complex, slowly progressive, and irreversible disorder of the brain involving multiple pathways and molecular mechanisms, yet to be clearly delineated.

Nonetheless, several therapeutic approaches have been considered, including (i) inhibition of either β- or γ-secretase that generate Aβ from βAPP (Ghosh and Osswald 2014; De Strooper et al. 2010; Ran et al. 2014); (ii) prevention of Aβ oligomerization or enhancing its clearance by active or passive immunization (Lambracht-Washington and Rosenberg 2013; Fettelschoss et al. 2014; Schenk et al. 1999); (iii) the use of metal chelators to avoid metal-induced rapid Aβ aggregation (Sharma et al. 2012; Squitti 2012); (iv) anti-inflammatory agents (Morales et al. 2014); (v) use of cholesterol-lowering drugs (Barone et al. 2014); and (vi) use of antioxidant agents (Aliev et al. 2008), among other therapeutics (http://www.alzforum.org/therapeutics). Unfortunately, no clinical proof has been provided yet that validates the amyloid cascade hypothesis (Mullane and Williams 2013). This makes preventive therapies a desirable aim in future clinical trials. No doubt, the Alzheimer's prevention (API) Colombia initiative, a plan to accelerate the evaluation of pre-symptomatic treatments in the "Paisa" community, might provide critical information for the advancing of AD treatments (Reiman et al. 2011; Ayutyanont et al. 2014; Kosik et al. 2015).

Aβ has naturally been at the center of therapeutic strategies (Aisen 2009; Bayer and Wirths 2014), and tau has recently been recognized as another important player in the neurodegenerative AD process. It is thus reasonable to assume that therapeutic approaches should be jointly directed against both amyloidogenic proteins (Boutajangout and Wisniewski 2014; Golde et al. 2011). Furthermore, due to the multifactorial nature of AD, we think that pleiotropic interventions are urgently needed for individuals at genetic risk of AD, as elegantly proposed by Frautschy and Cole (Frautschy and Cole 2010), especially for asymptomatic *PSEN 1* p.E280A mutation carriers. It is hoped that present and future development efforts may release not only the "Paisas" from Dr. Alzheimer's visit, but help the scientific community to further understand this fatidic *maladie*.

At present, there is as yet no definitive cure for AD. It is therefore imperative to answer two basic questions. First, what is (are) the molecular mechanism(s) responsible by which Aβ induces selective neural damage and death leading to brain dysfunction? Second, what are the potential therapeutic approaches that could be implemented to retard, prevent or interrupt the pathophysiological process in AD? Our approach to clarify these uncertainties involves the use of lymphocytes as a cell model, and *Drosophila melanogaster*, as in vivo model of AD.

2.3 βAdly Treated Lymphocytes: A Model of Oxidative Stress in Alzheimer's Neurons

Oxidatives stress (OS) refers to an imbalance in which free radicals (defined as any atom or molecule that has one or more unpaired electrons in its outer shells) and their products (e.g. superoxide anion radical, $O_2^{\bullet-}$; hydroxyl radical, •HO; sulfydryl radical, −S•; nitric oxide, NO•; lipid peroxy radical, LOO•) are in excess of enzymatic (e.g., catalase, CAT; glutathione peroxidase, GPx) and non-enzymatic defense (e.g. vitamin E & C) mechanisms. Chemically, free radicals and products, collectively known as reactive oxygen species (ROS), are predominantly generated by the transfer of one electron to oxygen (O_2) into superoxide ion ($O_2^{\bullet-}$). Addition of one more electron to $O_2^{\bullet-}$ will generate the peroxide ion (O_2^{2-}). The addition of $2H^+$ to

O_2^{2-} ion results in hydrogen peroxide (H_2O_2, a non-radical oxygen species) which decomposes rapidly into the more reactive hydroxyl radical, HO• in the presence of $O_2^{\bullet-}$ (Haber-Weiss reaction: $O_2^{\bullet-} + H_2O_2 + H^+ \rightarrow HO\bullet + O_2 + H_2O$) or metals (e.g. Fenton reaction: $Fe_2^+ + H_2O_2 + H^+ \rightarrow Fe_3^+ + HO\bullet + H_2O$). Because those ROS are capable of reacting with lipids, proteins, nucleic acids and other molecules, OS can lead to alterations in cells with an accumulation of oxidized products such as aldehydes and isoprostanes from lipid peroxidation, protein carbonyls from protein oxidation, and base adducts from DNA oxidation.

All of these chemical imprints of OS have been amply found in AD brain (Praticò 2008) and support the hypothesis that genetic alterations (e.g. over-production of Aβ(1–42) by *PSEN-1* mutations), environmental factors (e.g., Fe, Cu or Mn metal ions (Gaeta and Hider 2005)), diminished cerebral energy metabolism and excitotoxic events, working in a convergent or complimentary manner, induce OS to damage and kill AD neurons (Markesbery 1997). Indeed, recent evidence indicates that OS phenomenon is an early event and might have a pathological role in the pathogenesis of this disease (Nunomura et al. 2001).

Not surprisingly, Aβ has been suggested to play a central role in the OS damage in AD brain (Butterfield et al. 2001; Khan et al. 2006; Smith et al. 2007). Although the mechanism by which Aβ(1–42) generates ROS is not yet fully established, methionine (met) at position 35 (Met^{35}) of Aβ(1–42) (Fig. 2.2c) has been implicated as a critical amino acid for the OS and neurotoxic properties in AD brain (Butterfield and Sultana 2011; Butterfield et al. 2001). Most probably, the reduction of molecular O_2 by the sulfur-containing element of met^{35} yields $O_2^{\bullet-}$, which then undergoes spontaneous or enzyme-catalysed dismutation to H_2O_2 (Fig. 2.2d). Indeed, H_2O_2 is generated as a short burst early on in the aggregation process of amyloid peptides and British dementia peptide (Tabner et al. 2005). Furthermore, Behl et al. (1994) demonstrated that Aβ(1–42) causes increased levels of H_2O_2 to accumulate in cells and lipid peroxidation. Interestingly, they also found that Aβ(1–42) induces the activity of the nuclear factor-kappaB (NF-κB), a transcription factor thought to be regulated by OS. This last observation implicates Aβ(1–42) as being able to trigger oxidative signaling mechanisms involved in cell death.

Together, these data prompted us to test whether Aβ(1–42) might induce H_2O_2 generation, NF-κB activation and cytotoxicity in lymphocyte cells. Lymphocytes were selected as a model system based on their biochemistry, metabolic and physiological resemblance to neuronal cells (Jimenez-Del-Rio and Velez-Pardo 2012). In fact, lymphocytes not only express homologous neurochemical systems (e.g. catecholaminergic, serotonergic, cholinergic, glutamatergic, adrenergic systems) pertaining to neurons, but also express βAPP751 and βAPP770 mRNA isoforms (Ebstein et al. 1996), PSEN1 and PSEN 2 proteins (Mirinics et al. 2002; Takahashi et al. 2003), glucose transporters Glut 1 and 3 (Fu et al. 2004), and IGF-1 (Tapson et al. 1988; Kooijman et al. 1992), among others. Most importantly, lymphocytes and neurons are post-mitotic cells, i.e. in the G_0 phase of the cell cycle, and both cells express similar molecular death machinery. It is worth mentioning that lymphocytes have even been postulated as potential diagnostic and/or progression biomarkers of AD (Sultana et al. 2013; Song et al. 2012). Hence, peripheral blood lymphocytes are valid and important experimental tools for understanding the molecular signalling machinery and metabolic processes of cell death/survival in AD neurons.

Effectively, we have demonstrated that lymphocytes exposed to (10 μM) Aβ(25–35) (i.e. the cytotoxic functional sequence of the amyloid peptide, for a review see (Kaminsky et al. 2010)) induces apoptosis either alone or in the presence of (25 μM) iron in a concentration-dependent fashion by an OS mechanism (Velez-Pardo et al. 2002). Interestingly, Aβ(25–35) was able not only to generate H_2O_2 and induce NF-κB activation, but also induced p53 and c-Jun transcription factors, c-Jun N-terminal kinase

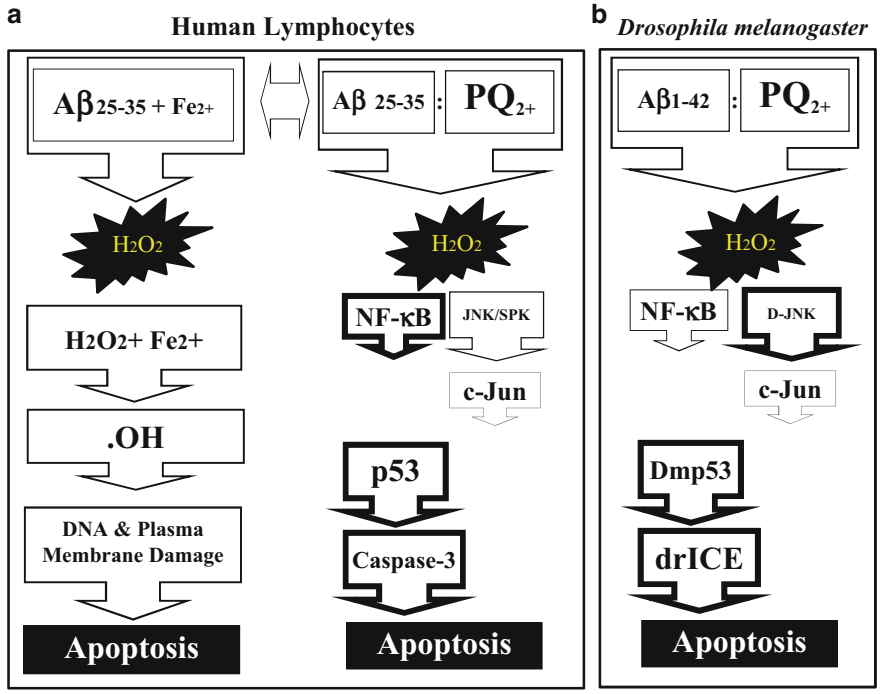


Fig. 2.3 Schematic model of Aβ(25–35) and Fe^{2+}-induced apoptosis by oxidative stress mechanism in peripheral blood lymphocytes (PBL) and *Drosophila melanogaster* neurons. (**a**) The Aβ(25–35) fragment alone or in combination with Fe^{2+} generates H_2O_2. The latter might activate in parallel NF-κB and JNK/SAPK pathways, which in turn activate the p53 and c-Jun transcription factors, respectively. P53 in turn activates the pro-apoptotic Bax protein, which induces cytochrome c release from mitochondria to activate the apoptosome complex. This complex activates caspase-3, leading PBL cells to cell death by apoptosis. Alternatively, H_2O_2 reacts with Fe^{2+} to produce highly reactive oxygen species (probably hydroxyl radicals by Fenton reaction), which directly damage nuclear DNA and provoke plasma membrane rupture leading to apoptosis. Noticeably, paraquat (PQ^{2+}) induced a similar mechanism in PBL. (**b**) PQ^{2+} induces a similar oxidative stress mechanism in *Drosophila melanogaster* neuronal cells (**a** is modified and reproduced with permission from Velez-Pardo et al. (2002))

(JNK) and caspase-3 activation, mitochondrial damage, chromatin condensation and nuclei fragmentation – all features typical of apoptosis (Fig. 2.3a). Not surpringly, specific inhibitors (e.g. pifithrin-α and Ac-DEVD-cho, inhibitors of p53 and caspase-3, respectively), antioxidant agents (e.g. *N*-acetyl-cysteine, the cannabinoids CP55940 and JHW-015) and growth factors (e.g. insulin growth factor-1) protected lymphocytes against Aβ(25–35)/H_2O_2-induced apoptosis (Jimenez Del Rio and Velez-Pardo 2006; Velez-Pardo and Del Rio 2006). These results imply that H_2O_2 generation and OS precede the apoptotic process, and that once it is generated, H_2O_2 is able to trigger apoptotic signaling (Marinho et al. 2014).

But then how does H_2O_2 induce apoptosis in lymphocytes? Althought H_2O_2 itself is a mild oxidant and is relatively inert with most biomolecules, it is able to oxidize cysteine residues in proteins to either cysteine sulfenic acid (Cys-SOH) or disulfide bond (Cys-S-S-Cys). This oxidative chemical process might change the quaternary structure of the target protein, thereby activating/inactivating it. In fact, it has been demonstrated that H_2O_2 is able to indirectly activate the NF-κB by at least three different mechanisms (Jung et al. 2008; Schoonbroodt et al. 2000). Once NF-κB is freed from inhibition, the p50/p65 active dimeric protein translocates into the nucleus and transcribes either anti-apoptotic (e.g. *Bcl-2*, *cIAP-1 & 2*, *Bcl-xL*) or

pro-apoptotic (e.g. *p53*) genes. At this point, a vicious cycle is established wherein p53 plays a critical role by balancing the cell to a death decision because of its many actions. First, p53 transcribes pro-apoptotic genes such as *Bax*, which in turn contribute to the permeabilization of the outer mitochondrial membrane by antagonizing anti-apoptotic proteins (e.g., Bcl-2, cIAP-1-2, Bcl-xL). Second, p53 not only induces pro-oxidant genes (e.g., *p53-induced gene-3*, *proline oxidase*), which generate more H_2O_2, but also represses the transcription of antioxidant genes (e.g., *phosphoglycerate mutase*, *NAD(P) H: quinone oxidoreductase-1*) (Olovnikov et al. 2009). Elevated stress stimuli by H_2O_2 production and further activation of NF-κB induce up-regulation of pro-apoptotic genes (e.g., *p53*), which in turn amplify the initial H_2O_2-induced cell death signal. Formation of the mitochondrial permeabilization transition pore allows the release of apoptogenic proteins, by a not fully established mechanism, such as the apoptosis-inducing factor (AIF, (Norberg et al. 2010)) responsible for DNA fragmentation and chromatin condensation (i.e., stage I nuclei morphology) and cytochrome *c*, which together with Apaf-1, dATP, and procaspase-9 (i.e., the apoptosome) elicits caspase-3 protease activation (Zou et al. 1999). This protease is essential for the fragmentation and morphological changes associated with apoptosis (i.e., stage II nuclei morphology (Jänicke et al. 1998)). H_2O_2 is also able to induce the apoptosis signal-regulating kinase (ASK1) activation by a mechanism that involves oxidation and release of thioredoxin from ASK-1 complex (Saitoh et al. 1998). Once activated, ASK-1 phosphorylates MKK4/MAPK kinase, which in turn phosphorylates c-Jun N-terminal kinase (JNK)/stress apoptotis protein kinase (SAPK) (Yang et al. 1997). JNK/SAPK signals cell death either via c-Jun activation (Gupta et al. 1996) or via stabilization and/or activation of p53 (Oleinik et al. 2007; Fuchs et al. 1998). Importantly, JNK/SAPK can also phosphorylate tau protein *in vitro* (Goedert et al. 1997; Reynolds et al. 1997). In agreement with others, this last observation suggests that tangle pathology is a downstream process related to Aβ signalization, consistent with the amyloid cascade hypothesis (Götz et al. 2001; Oddo et al. 2003; LaFerla 2010).

Is there a link between Aβ-induced apoptosis in lymphocytes and AD brain pathology? It would be possible to answer this question affirmatively, if one considers that Aβ-induced OS and most probably apoptosis in (*in vivo*) model AD brain (LaFerla et al. 1995; Selznick et al. 1999) and (*in situ*) human post-mortem AD brains (Selznick et al. 1999; Su et al. 2001; Zhao et al. 2003) occurs by similar mechanisms as those shown in Aβ-induced toxicity (*in vitro*) in lymphocytes. Of note, Garcia-Ospina et al. (2003) showed a correlation between DNA damage, detected by TdT-mediated dUTP Nick End Labeling (TUNEL) technique, with a positive detection of c-Jun, NF-κB, p53 and Par-4 transcription factors in post-mortem *PSEN-1* E280A AD brains. Further, it has been directly demonstrated that a subset of amyloid plaques produce ROS in living, Alzheimer's models (e.g., Tg2576 APP overexpressing transgenic mice) and in human post-mortem Alzheimer tissue (McLellan et al. 2003). Huang and co-workers found NF-κB precursor p105 and IκBγ inhibitor protein elevated in AD brain (Huang et al. 2005). Likewise, activation of p53 (Cenini et al. 2008), caspase-3 (Su et al. 2001), c-Jun and JNK (Thakur et al. 2007; Shoji et al. 2000), AIF (Yu et al. 2010; Lee et al. 2012) and DJ-1, an antioxidant and OS sensor protein (Choi et al. 2006; Baulac et al. 2009), have been shown in AD brains. Collectively, these findings further support the notion that in AD brain either β-plaques or Aβ oligomers may induce cell death by a molecular signalling process similar to that found in our non-neuronal model exposed to Aβ. Indeed, increased OS, elevated apoptosis and decreased basal mitochondrial membrane potential levels, as well as enhanced sensitivity to different complex inhibitors of the respiratory chain, have all been reported in lymphocytes from AD patients (reviewed by Leuner et al. (2012)). Although other investigators have proposed complex disease pathways in AD pathogenesis (e.g. Crouch et al. 2008; Bettens et al. 2010), the Aβ-induced toxicity in lymphocytes should be interpreted as

a proposal for a minimal mechanism of cell death signaling induced by OS to explain neuronal demise.

Interestingly, we found that Aβ(25–35)-induced cytotoxicity can be recapitulated by paraquat (PQ), a mitochondrial redox cycling compound. Indeed, PQ induced a similar OS cell death mechanism in lymphocytes (Fig. 2.3a, Rio and Velez-Pardo 2008). Cannabinoids and growth factor protect and rescue lymphocytes against both Aβ and PQ exposure (Rio and Velez-Pardo 2008). Hence, it may be concluded that although the initiator cytotoxic molecules are clearly different in nature, they evidently converge on a common activator compound and pathway, namely the H_2O_2 signaling molecule that triggers an OS-mediated apoptotic mechanism (Jimenez-Del-Rio and Velez-Pardo 2004, 2012).

In conclusion, lymphocytes have been useful in deciphering molecular signaling events in AD. Nonetheless, they have to be viewed as a limited model for the study of other important aspects of the disease. For instance, lymphocytes do not express tau protein (Herrera-Rivero et al. 2013). Therefore, they provide no clues as to how Aβ interacts with such a protein. Furthermore, as a model system, lymphocytes are limited to answer questions in relation to Aβ-induced alterations of higher functions such as locomotion, memory and learning process. To address this problem, we changed our focus to the fruitfly *Drosophila melanogaster*. In the next section, we discuss the advantages of using the fly to model AD, and the fact that PQ might replace Aβ to further advance our understanding of the molecular mechanism of neuronal death in AD.

2.4 Next Patient: *Drosophila melanogaster*

In 1933, Thomas Hunt Morgan was awarded the Nobel Prize "*for his discoveries concerning the role played by the chromosome in heredity*" using *Drosophila melanogaster* as a genetics model. Since then, several other scientists have been awarded this prominent prize for discoveries in the fields of genetics (Hermann Muller, 1946), development (Edward B. Lewis, Christiane Nusslein-Volhard and Eric F. Wieschaus, 1995), odour/olfactory systems (Richard Axel and Linda Buck, 2004) and immunity (Jules Hoffman, Bruce Beutler and Ralph Steinmann, 2011). What is it about flies that make them particularly useful to study Alzheimer's disease? From an evolutionary standpoint, *Drosophila* conserves several AD-related pathophysiological systems. To start with, basic local alignment search (BLAST) comparison between human and *Drosophila* proteins involved in memory processes showed high identity and similarity homology (Table 2.1). Strikingly, the fundamental molecular mechanisms implicated in implicit and explicit memory storage work similary in *Drosophila* as well as in *Aplysia* and mammals (see Frank and Greenberg 1994; Silva and Murphy 1999; Skoulakis and Grammenoudi 2006; Barco et al. 2006; Kandel 2012; Kandel et al. 2014, among others). The fly has a complex nervous system that consists of 100,000 neurons, which includes a subset of 200 neurons that contain dopamine and serotonin neurotransmitters involved in memory processes (Waddell 2010; Berry et al. 2012; Sitaraman et al. 2012). Moreover, the fly possesses 2,500 Kenyon neuron cells and axons (i.e. α/β, α'/β', γ lobes) constituting the mushroom bodies (MB), which are anatomical structures analogous to the human hippocampus (Campbell and Turner 2010), and central complex ellipsoid bodies involved in olfactory memory (Perisse et al. 2013a) and visual place learning and memory. Consequently, the fly can be trained to learn and perform complex behaviours requiring short- and long-term memory tasks (Ali et al. 2011; Ofstad et al. 2011; Perisse et al. 2013b; Pitman et al. 2009; Foucaud et al. 2010). Conclusively, research with *Drosophila* has provided essential information to enhance our understanding of human memory (Chakraborty et al. 2011) and early-onset cognitive disorders (Oortveld et al. 2013; van der Voet et al. 2014).

Notably, APP orthologs have been identified too in *Drosophila* (Rosen et al. 1989; Luo et al. 1992), and their processing is also evolutionarly preserved (Tharp and Sarkar 2013,

Table 2.1 Comparison of amino acid sequences of proteins involved in learning and memory, amyloid-β processing protein, cell death (apoptosis) and metal metabolism by basic local alingment search tool (BLAST) in *Homo sapiens* and *Drosophila melanogaster*

System	Protein name	*Homo sapiens*	Accession number	*Drosophila melanogaster*	Accession number	BLAST result: Identity, %; Similarity, %; (E)
Learning and memory	Ca(2+)/ calmodulin-activated adenylyl cyclase	Adenylate cyclase type 1	Q08828.2	Ca(2+)/ calmodulin-responsive adenylate cyclase	P32870.2	46 %; 64 %; (1×10^{-112})
		Adenylate cyclase type 8	P40145.1	Ca(2+)/ calmodulin-responsive adenylate cyclase	P32870.2	58 %; 76 %; (6×10^{-139})
	Protein kinase A	PKA alpha (betta)	P17612.2	PKA c1 Isoform B (C,D)	AAN10703.1	82 %; 89 %; (0.0)
		PKA gamma	P22612.3 P22612.3 P22612.3	PKA c1 Isoform B (C,D)	AAN10703.1	75 %; 85 %; (0.0)
	Protein kinase C	PKC epsilon	CAA46388.1	PKC	P13678.1	60 %; 74 %; (0.0)
		PKC alpha	EAW89014.1	PKC	P13678.1	63 %; 79 %; (8×10^{-154})
		PKC gamma	EAW72161.1	PKC	P13678.1	44 %; 57 %; (8×10^{-144})
		PKC eta	AAH37268.1	PKC	P13678.1	55 %; 68 %; (0.0)
		PKC beta	AAH36472.1	PKC	P13678.1	63 %; 70 %; (1×10^{-153})
		PKC iota	AAB17011.1	PKC	P13678.1	43 %; 57 %; (3×10^{-136})
		PKC zeta	AAA36488.1	PKC	P13678.1	43 %; 60 %; (6×10^{-135})
		PKC theta	AAI13360.1	PKC	P13678.1	46 %; 62 %; (0.0)
		PKC mu	CAA53384.1	PKC	P13678.1	30 %; 49 %; (6×10^{-31})
	Protein phosphatase 1	Protein phosphatase-1	AAA36508.1	Protein phosphatase1	CAA39820.	88 %; 92 %; (0.0)
	Ubiquitin hydroxylase	Ubiquitin carboxyl-terminal hydrolase isozyme L1	P09936.2	Ubiquitin carboxyl-terminal hydrolase 36	Q9VRP5.3	50 %; 70 %; (8.9)
	Phospholipase C	PLC beta1	AAF86613.1	Phospholipase C	AAA28820.1	50 %; 68 %; (1×10^{-140})
		PLC beta2	AAP35551.1	Phospholipase C	AAA28820.1	41 %; 60 %; (2×10^{-48})
		PLC beta3	CAA85776.1	phospholipase C	AAA28820.1	46 %; 63 %; (0.0)
		PLC beta4	AAI43869.1	Phospholipase C	AAA28820.1	46 %; 64 %; (3×10^{-128})
		PLC delta1	AAA73567.1	Phospholipase C	AAA28820.1	46 %; 59 %; (1×10^{-58})

(continued)

Table 2.1 (continued)

System	Protein name	*Homo sapiens*	Accession number	*Drosophila melanogaster*	Accession number	BLAST result: Identity, %; Similarity, %; (E)
		PLC delta3	AAH72384.1	phospholipase C	AAA28820.1	35 %; 52 %; (2×10^{-55})
		PLC delta4	AAH06355.1	Phospholipase C	AAA28820.1	45 %; 61 %; (4×10^{-63})
		PLC epsilon1	AAI51855.1	Phospholipase C	AAA28820.1	42 %; 58 %; (6×10^{-43})
		PLC gamma1	AAI44137.1	Phospholipase C	AAA28820.1	44 %; 58 %; (6×10^{-47})
		PLC gamma2	AAQ76815.1	Phospholipase C	AAA28820.1	34 %; 50 %; (2×10^{-49})
		PLC eta	AAI13951.1	Phospholipase C	AAA28820.1	29 %; 46 %; (5×10^{-88})
		PLC zeta	AAN71895.1	Phospholipase C	AAA28820.1	38 %; 56 %; (4×10^{-53})
	CCAAT-box-enhanced binding protein	C/EBP gamma	AAC50201.1	C/EBP	AAA28415.1	40 %; 73 %; (4×10^{-15})
		C/EBP delta	EAW86679.1	C/EBP	AAA28415.1	40 %; 70 %; (2×10^{-16})
		C/EBP beta	EAW75629.1	C/EBP	AAA28415.1	44 %; 68 %; (1×10^{-16})
		C/EBP épsilon	EAW66183.1	C/EBP	AAA28415.1	43 %; 64 %; (1×10^{-15})
		C/EBP zeta	AAH34475.1	C/EBP	AAA28415.1	34 %; 48 %; (0.078)
	Ca2+/ Calmodulin protein kinase II-α	CaMKII-α	AAH40457.1	CaM-kinase II alpha	Q00168.1	71 %; 81 %; (0.0)
	Calcineurin	Calcineurin	AAC37581.1	Calcineurin A1, isoform A (D)	AAF57105.3	24 %; 48 %; (1.8)
		Calcineurin	AAC37581.1	Calcineurin B, isoform A (B)	AAF46026.1	36 %; 50 %; (4.6)
	Cytoplasmic polyadenylation element binding protein	CPEB1	Q9BZB8.1	orb2, isoform A (B,C,D, H)	AAF50352.1	42 %; 59 %; (2×10^{-63})
		CPEB2,3,4	Q7Z5Q1.3	orb2, isoform A (B,C,D,H)	AAF50352.1	88 %; 93 %; (2×10^{-174})
	NMDA receptor	NMDA	AAA21180.1	dNR 1	AAF52016.1	48 %; 67 %; (0.0)
		NMDA	AAA21180.1	dNR 2, isoform A (B,C,D,E,F,G)	AAN09051.2	29 %; 47 %; (3×10^{-91})

(continued)

Table 2.1 (continued)

System	Protein name	*Homo sapiens*	Accession number	*Drosophila melanogaster*	Accession number	BLAST result: Identity, %; Similarity, %; (E)
	AMPA receptor	AMPA1	P42261.2	Glutamate receptor IA	AAF50652.2	40 %; 57 %; (0.0)
		AMPA1	P42261.2	Glutamate receptor IB, isoform A (B,C)	AAF50306.2	36 %; 53 %; (2×10^{-114})
		AMPA2	P42262.3	Glutamate receptor IA	AAF50652.2	42 %; 58 %; (0.0)
		AMPA2	P42262.3	Glutamate receptor IB, isoform A (B,C)	AGB94317.1	36 %; 53 %; (2×10^{-114})
		AMPA3	P42263.2	Glutamate receptor IA	AAF50652.2	42 %; 58 %; (0.0)
		AMPA3	P42263.2	Glutamate receptor IB, isoform A (B,C)	AAF50306.2	36 %; 52 %; (2×10^{-120})
		AMPA4	P48058.2	Glutamate receptor IA	AAF50652.2	43 %; 60 %; (0.0)
		AMPA4	P48058.2	Glutamate receptor IB, isoform A (B,C)	AAF50306.2	42 %; 56 %; (0.0)
	Serotonin receptor	Serotonin receptor	CAA05851.1	Serotonin receptor 1	P20905.1	83 %; 100 %; (1.5)
		Serotonin receptor	AAA66493.1	Serotonin receptor 2A	P28285.2	48 %; 69 %; (3×10^{-61})
		Serotonin receptor	AAA66493.1	Serotonin receptor 2B	P28286.3	48 %; 68 %; (3×10^{-62})
	Dopamine receptor	Dopamine receptor D1A	P21728.1	Dopamine receptor	AAC47161.1	35 %; 48 %; (6×10^{-59})
		Dopamine receptor D1B(5)	CAA41360.1	Dopamine receptor	AAC47161.1	42 %;57 %; (4×10^{-40})
		Dopamine receptor D2	P14416.2	Dopamine receptor	AAC47161.1	34 %; 49 %; (1×10^{-62})
		Dopamine receptor D3	P35462.2	Dopamine receptor	AAC47161.1	36 %; 50 %; (3×10^{-65})
		Dopamine receptor D4	P21917.2	Dopamine receptor	AAC47161.1	40 %; 54 %; (8×10^{-26})
		Dopamine receptor D1A	P21728.1	Dopamine receptor	CAA54451.1	40 %; 55 %; (6×10^{-71})
		Dopamine receptor D1B(5)	CAA41360.1	Dopamine receptor	CAA54451.1	36 %; 52 %; (3×10^{-70})

(continued)

Table 2.1 (continued)

System	Protein name	*Homo sapiens*	Accession number	*Drosophila melanogaster*	Accession number	BLAST result: Identity, %; Similarity, %; (E)
		Dopamine receptor D2	P14416.2	Dopamine receptor	CAA54451.1	36 %; 56 %; (2×10^{-35})
		Dopamine receptor D3	P35462.2	Dopamine receptor	CAA54451.1	33 %; 46 %; (3×10^{-52})
		Dopamine receptor D4	P21917.2	Dopamine receptor	CAA54451.1	38 %; 57 %; (6×10^{-24})
	Tyrosine hydroxylase	Tyrosine hydroxylase	AAI43612.1	Tyrosine hydroxylase	CAA53802.1	54 %; 74 %; (5×10^{-168})
APP	APP	APP_{770}	P05067.3	Appl	AAD55414.1	(**36 %**) (**51 %**) (8×10^{-37})
		APP_{751}	NP_958816.1	Appl	AAD55414.1	(**36 %**) (**51 %**) (9×10^{-37})
		APP_{695}	NP_958817.1	Appl	AAD55414.1	(**36 %**) (**51 %**) (2×10^{-37})
	Aβ42	Amyloid beta-peptide	1IYT_A	–	–	–
		(1–42)	P05067.3			
	PSEN	PS1	P49768.1	PS-D A (B-E)	AAF51598.1	53 %; 66 %; (5×10^{-145})
		PS2	AAP35630.1	PS-D A (B-E)	AAF51598.1	53 %; 67 %; (8×10^{-110})
	Nicastrin	Nicastrin-1	NP_056146.1	Nicastrin-A (C-E)	AAF56349.2	31 %; 48 %; (8×10^{-90})
		Nicastrin-2	NP_001277113.1	Nicastrin-A (C-E)	AAF56349.2	30 %; 48 %; (9×10^{-90})
		Nicastrin-3	NP_001277115.1	Nicastrin-A (C-E)	AAF56349.2	30 %; 46 %; (1×10^{-38})
	APH-1	APH-1A	Q96BI3.1	dAPH-1A	AAF51212.1	47 %; 66 %; (2×10^{-69})
		APH-1B	Q8WW43.3	dAPH-1B	AGB92500.1	44 %; 60 %; (5×10^{-59})
	PEN-2	PEN-2	Q9NZ42.1	pen-2	Q86BE9.3	61 %;74 %; (3×10^{-45})
	BACE	BACE-1	P56817.2	dBACE	NP_609235.1	26 %;42 %; (5×10^{-30})
		BACE-2	Q9Y5Z0.1	dBACE	NP_609235.1	29 %; 44 %; (2×10^{-30})
	α-Secretase	ADAM10	O14672.1	Kuzbanian	P07207.3	28 %; 41 %; (1.1)
	MAPT	MAPT	P10636.5	dTau	AAK54456.1	38 %; 56 %; (6×10^{-33})

(continued)

Table 2.1 (continued)

System	Protein name	*Homo sapiens*	Accession number	*Drosophila melanogaster*	Accession number	BLAST result: Identity, %; Similarity, %; (E)
Apoptosis	ASK-1	ASK-1	Q99683.1	DASK-1	BAC16514.1	42 %; 58 %; (0.0)
	MEKK-1	MEKK-1	Q13233.4	D-MEKK-1	AAF55592.3	34 %; 54 %; (1×10^{-45})
		MEKK-1	Q13233.4	B-MEKK-1	AAN13787.**1**	34 %; 54 %; (1×10^{-45})
	MKK4	MKK4	P45985.1	MKK4-A (B,C)	AAF54258.1	67 %; 78 %; (7×10^{-153})
	IKK	IKK α	p25963	Cactus	AAA85908	37 %; 51 %; (1×10^{-28})
		IKK β	Q15653	Cactus	AAA85908	30 %; 45 %; (5×10^{-19})
	NF-κB	NF-κB 1 (p50)	P19838	RELISH	AAF20138	34 %; 54 %; (2×10^{-44})
		NF-kB 1 (p50)	P19838	Dorsal	AAQ65068	39 %; 53 %; (1×10^{-47})
		NF-kB 1 (p50)	P19838	Dif	EDW90181	33 %; 48 %; (3×10^{-38})
		NF-kB 1 (p65)	Q04206.2	RELISH	AAF20138	36 %; 53 %; (1×10^{-41})
		NF-kB 1 (p65)	Q04206.2	Dorsal	AAQ65068	49 %; 61 %; (1×10^{-75})
		NF-kB 1 (p65)	Q04206.2	dif	EDW90181	36 %; 56 %; (6×10^{-62})
	JNK	JNK-1/MAPK8	AAI30571.1	dJNK (Basket)	P92208.1	80 %; 88 %; (0.0)
	c-JUN	c-JUN	CAG46552	d-Jun	CAA73154	55 %; 76 %; (1×10^{-22})
	P-53	p-53	AAC12971	Dmp-53	AAF75270	24 %; 42 %; (5×10^{-6})
	BAX	BAX	AAB35593.1	Drob-1	BAA89603.2	67 %; 66 %; (3.0)
	Cyto C	Cyto-C	AAA35732	Cyto c-A (B)	AAF55946.1	40 %; 40 %; (6.4)
	Caspase-9	Caspase-9	BAA87905.1	Dronc	AAF50180.1	33 %; 54 %; (1×10^{-13})
	Caspase-3	Caspase-3	CAC88866	drICE	CAA72937	42 %; 60 %; (1×10^{-46})
	DFF45	DFF45	O00273.1	dICAD-A (B)	AAF58589.1	35 %; 48 %; (2×10^{-9})
	DFF40	DFF40	BAA32250.1	dCAD	BAA97120	28 %; 43 %; (4×10^{-30})
	APAF-1	APAF-1	O14727.2	Dark: Apaf1-A (B)	AAM68488.1	23 %; 44 %; (3×10^{-9})
	AIF	AIF	AAD16436.1	AIF-A (B,C)	AAF51299.2	52 %; 68 %; (8×10^{-175})

(continued)

Table 2.1 (continued)

System	Protein name	*Homo sapiens*	Accession number	*Drosophila melanogaster*	Accession number	BLAST result: Identity, %; Similarity, %; (E)
Metal metabolism	Ferritin	Ferritin heavy chain	NP_002023.2	Ferritin 1	AAF57034.1	37 %; 52 %; (5×10^{-28})
		Ferritin light chain	NP_000137.2	Ferritin 2	AAF57038.1	24 %; 43 %; (1×10^{-11})
	Transferrin	Transferrin	ABI97197.1	Transferrin-1	NP_523401.2	26 %; 41 %; (2×10^{-28})
		Transferrin	ABI97197.1	Transferrin-2	AAF49900.1	34 %; 49 %; (2×10^{-53})
		Transferrin	ABI97197.1	Transferrin-3	AAF58039.1	23 %; 38 % (1×10^{-27})
	Divalent metal ion transportin	DMT-1	P49281.2	Malvolio	NP_732584.1	61 %; 76 %; (0.0)
	CTR-1	hCTR-1	O15431.1	Ctr1A	AAF46182.3	49 %; 61 %; (9×10^{-48})
		hCTR-1	O15431.1	Ctr1B	AAF54223.1	35 %; 51 %; (1×10^{-30})
	ATP7	ATP7A (B)	Q04656.3	DmATP7	AAF48104.3	47 %; 62 %; (0.0)

Abbreviations: *AMPA* α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (receptor), *APP* amyloid-β precursor protein, *APH-1* anterior pharynx-defective 1, *AIF* apoptosis inducing factor, *APAF* apoptosis protease-activating factor-1, *ASK-1* apoptosis signal-regulating kinase 1, *BAX* BCL2-associated X protein, *BACE* beta-site amyloid precursor protein cleaving enzyme 1, *CAD* Caspase Activated DNase, *JNK* c-Jun N-terminal kinases, *CTR-1* Copper transporter 1, *DIF* differentiation-inducing factor-1, *DFF* DNA fragmentation factor, *DRONC Drosophila melanogaster* NEDD2-like caspase, *DARK Drosophila* Apaf-1-related killer, *drICE Drosophila* ICE (Interleukin-1β-converting enzyme), *ICAD* Inhibitor of Caspase Activated DNase, *IKK* IκB kinase, *MEKK* MAPK/ERK kinase, *MAPT* microtubule-associated protein tau, *MKK* Mitogen-activated protein kinase kinase, *NMDA* N-methyl-D-aspartate (receptor), *NF-κB* nuclear factor-kappaB, *PEN-2* presenilin enhancer 2

Fig. 2.2b). In fact, several reports have shown that *in vivo* reconstitution of the γ-secretase complex (i.e. fly PSEN, NCT, APH-1 and PEN-2 proteins) has biochemical properties and a subcellular distribution resembling those of endogenous γ-secretase (Hu and Fortini 2003; Stempfle et al. 2010). Additionally, *Drosophila* express α-secretase-like protein encoded by the *kusbanian* gene (Rooke et al. 1996), and BACE-1 like secretase acitivity (Greeve et al. 2004). Although there is a lack of amino acid sequence conservation between the human β-peptide and that of the fly (Fig. 2.2b), Carmine-Simmen et al. (2009) have shown that Aβ fragments derived from the *Drosophila* orthologue APPL aggregate into intracellular fibrils, amyloid deposits, and cause age-dependent behavioral deficits and neurodegeneration. Most importantly, it was shown that the behavioral phenotypes precede extracellular amyloid deposit formation, supporting results that intracellular Aβ plays a key role in AD (Crowther et al. 2005). Therefore, *Drosophila* provides a comparable neurotoxic peptide effect from fly APPL. Additionally, several *Drosophila* AD models that express the toxic human Aβ42 have shown amyloid deposits, learning and memory deficiences, locomotor impairment, and premature death (Jahn et al. 2011) (for review, see refs. Iijima-Ando and Iijima 2010; Prüßing et al. 2013). Lately, Mhatre et al. (2014) have characterized a novel model of AD using *Drosophila melanogaster*, where they expressed the human AD-associated proteins APP and BACE in the central nervous system of the fly and showed synaptic defects in the larval neuromuscular junction (NMJ) leading to defective larval locomotion behavior, decreased presynaptic connections, altered mitochondrial

localization in presynaptic motor neurons and decreased postsynaptic protein levels. Most importantly, treatment of this model with the γ-secretase inhibitor L-685,458 suppressed the behavioral defects as well as the pre- and postsynaptic defects. These data comply with the notion that Aβ provokes early neuronal damage/alteration prior to Aβ aggregation and/or deposition. Based on such an assumption, one may wonder whether AD can be considered as a neurodevelopmental disorder rather than a neurodegenerative disease. Further investigation is needed to fully answer this question.

Another relevant point is that the apoptosis machinery is conserved in *Drosophila* (Abdelwahid et al. 2011, and Table 2.1). Despite the fact that Aβ induces neuronal cell death by activation of JNK (Troy et al. 2001; Tare et al. 2011), and causes mitochondrial dysfunction through OS (Abramov et al. 2004; Pagani and Eckert 2011; Spuch et al. 2012), no information is available concerning the role of p53 and other ortolog genes. Since PQ induces a similar molecular cascade of cell death as Aβ (Rio and Velez-Pardo 2008; Velez-Pardo et al. 2002) and *Drosophila* is amenable to ectopic gene knockdown or constitutive gene overexpression, we made use of the bipartite GAL4/*Upstream Activation Sequence* (UAS) system (Phelps and Brand 1998; Elliott and Brand 2008) to demonstrate that *Dmp53* (i.e. p53), *basket* (JNK) and *drICE* (caspase-3) gene knockdown in dopaminergic neurons (Fig. 2.4a–j) prolong life span and locomotor activity in *Drosophila melanogaster* lines chronically exposed to PQ, compared to untreated transgenic fly lines (Fig. 2.4h–j, Ortega-Arellano et al. 2013). In contrast, overexpression of *basket* and *Dmp53* flies sensitizes the transgenic flies to PQ treatment (Fig. 2.4k–m). It is anticipated that overexpression of Aβ might activate both *Dmp53* and *drICE*, leading to mitochondrial damage and contributing to neuronal death in *Drosophila* brain (Fig. 2.3c). It is therefore reasonable to suppose that Aβ might be able to trigger activation of *Dmp53*, *basket* and *drICE* via $O_2^{\bullet -}/H_2O_2$ signaling and cell death in a similar fashion as proposed in mammalian cells (Fig. 2.3a, b and Jimenez-Del-Rio and Velez-Pardo 2012). Finally, *Drosophila* expresses similar protein sequences involved in iron and copper metabolism such as ferretin, transferrin, human high-affinity copper importer Ctr1, and divalent metal ion transporter (DMT-1) that may modify Aβ-induced neurotoxicity (Table 2.1). Effectively, overexpressing the subunits of the iron-binding protein ferritin can rescue the toxicity of the Aβ peptide in *Drosophila* AD model system (Rival et al. 2009). It has also been found that inhibition of Ctr1 or overexpression of the copper exporter DmATP7 reduce copper accumulation in the brains of the flies, ameliorate neurodegeneration, enhance climbing ability, and prolong lifespan (Lang et al. 2013). Therefore, iron and copper might play a critical role in AD pathophysiology (Greenough et al. 2013).

Interestingly, Iijima et al. (2010) have shown that co-expression of Aβ1-42 with tau using the pan-neuronal retinal *glass multiple reporter* (gmr) promoter-GAL4 significantly enhanced the reduction in the external size of eyes and internal retina thickness compared to Aβ(1–42) expression alone, which did not significantly affect eye structures at this level of expression. Similarly, co-expression of Aβ(1–42) and tau using the pan-neuronal *embryonic lethal*, *abnormal vision* reporter (elav) promoter-GAL4 caused a complete loss of calyx (a dendritic region in the mushroom body) structures, whereas a normal calyx structure was observed in flies expressing Aβ(1–42) alone. Furthermore, phosphorylation of tau Ser^{262} was found critical for Aβ(1–42)-induced tau toxicity. In aggrement with these findings, Folwell and co-workers (2010) report that co-expression of Aβ(1–42) with tau (wild-type) increases tau phosphorylation and exacerbates tau-mediated phenotypes (i.e. neuronal dysfunction by disrupting axonal transport and synaptic structure resulting in behavioural impairments and reduced lifespan). Altogether, these results indicate that tau enhances Aβ-induced toxicity in *Drosophila* brain neurons, and reinforce the notion that tau – pathology is a downstream process related to Aβ signalization (Ploia et al. 2011; Chabrier et al. 2012). Based on the above observations, *Drosophila* seems to be an extremely useful and valid *in vivo* model

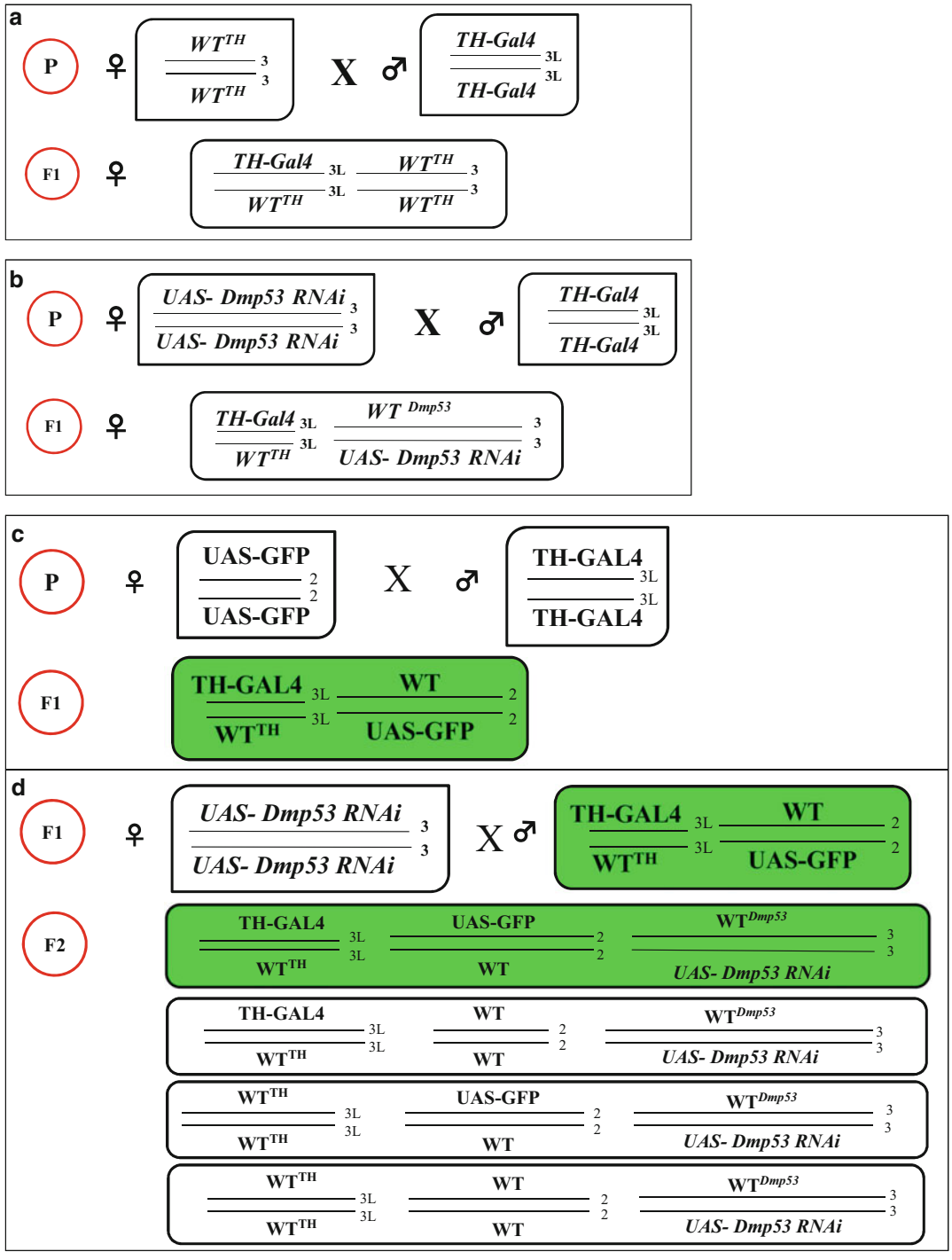


Fig. 2.4 (**a**) Scheme for basic fly cross and selection. *TH-Gal4*$^{+/-}$ flies were obtained by crossing *TH-Gal4*$^{+/+}$ (males, $n = 10$) and wild type Canton S *Drosophila melanogaster* (females, $n = 10$). After five days of husbandry, parental flies were discarded from the mating tubes. F1 flies were then reared according to standard procedures. (**b**) *TH-Gal4*$^{+/-}$, *UAS-Dmp53RNAi*$^{+/-}$ flies were obtained by crossing *TH-Gal4*$^{+/+}$ (males, $n = 10$) and *UAS-Dmp53RNAi*$^{+/+}$ (females, $n = 10$). (**c**) F1 *TH-Gal4*$^{+/-}$; *UAS-GFP*$^{+/-}$-flies were obtained by crossing *TH-Gal4*$^{+/+}$ (males, $n = 10$) and *UAS-GFP*$^{+/+}$ *Drosophila melanogaster* (females, $n = 10$).

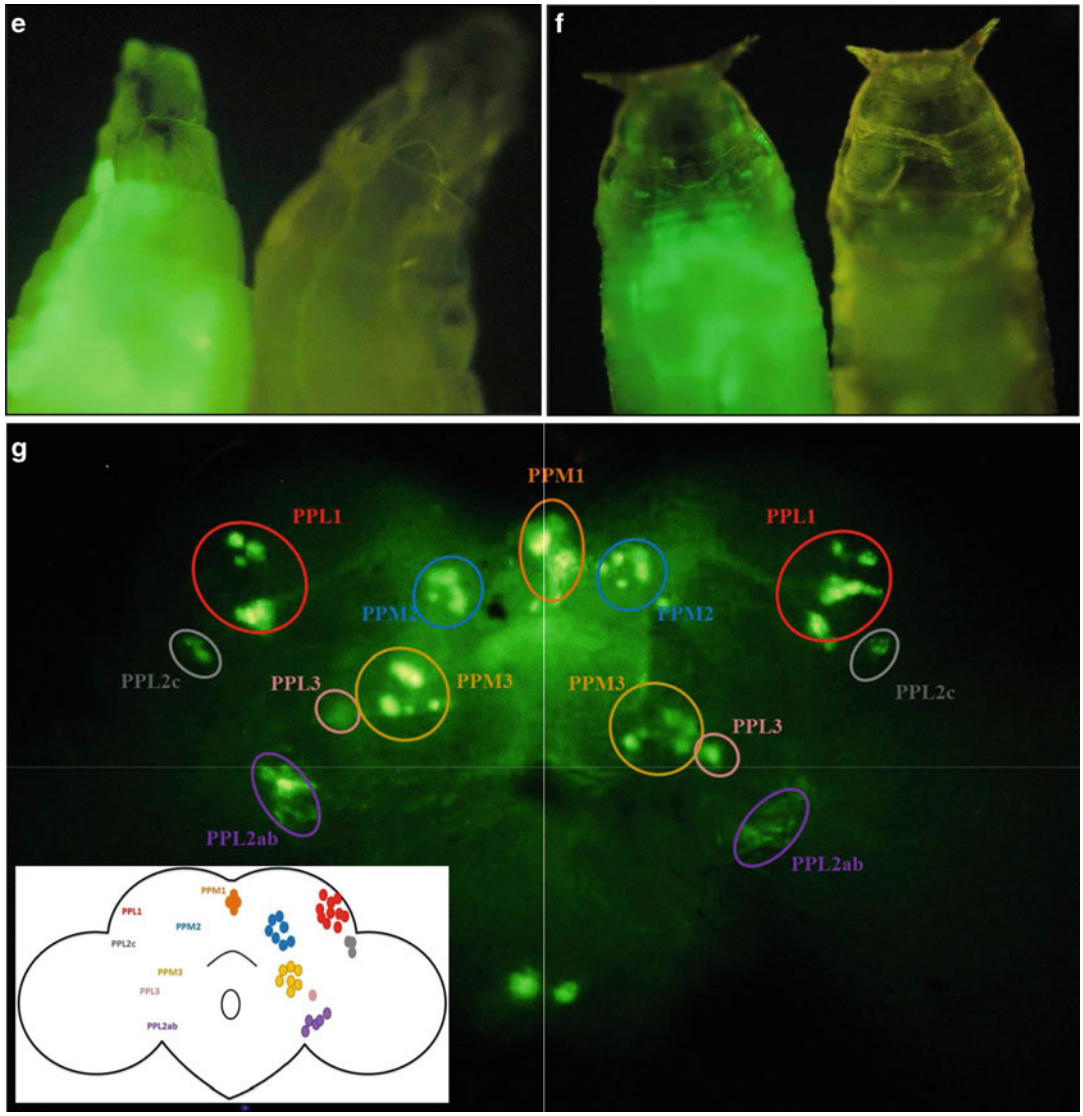


Fig. 2.4 (continued) (**d**) Males F1 *TH-Gal4*$^{+/-}$; *UAS-GFP*$^{+/-}$ were crossed with females *UAS-Dmp53 RNAi*$^{+/+}$ to obtain F2, where 1/4 represent *TH-Gal4*$^{+/-}$-*UAS-GFP*$^{+/-}$; *UAS-Dmp53 RNAi*$^{+/-}$. (**e**, **f**) Negative (translucent) and positive (green fluorescent) *TH-Gal4*$^{+/-}$-*UAS-GFP*$^{+/-}$; *UAS-Dmp53 RNAi*$^{+/-}$ larvae (**e**) and pupae (**f**). (**g**) Illustrates six clusters on the posterior side in whole mount of dissected brain from untreated *TH-GAL4*$^{+/-}$; *UAS-GFP*$^{+/-}$ flies: PPM1 (unpaired), PPM2 (paired), PPM3 (paired) (protocerebral posterior medial); PPL1, PPL2ab, PPL2c and PPL3 (paired) (protocerebral posterolateral). (**h–m**) Effect of paraquat (PQ) on the survival (**h**, **k**), locomotor activity (**i**, **l**) and dopaminergic neurons (**j**, **m**) knockdown (**h–j**, *TH-GAL4*$^{+/-}$; *UAS-Dmp53 RNAi*$^{+/-}$) or overexpression (**k–m**, *TH-GAL4*$^{+/-}$; *UAS-Dmp53*) in *Drosophila melanogaster*. *TH-GAL4*$^{+/-}$; *UAS-Dmp53 RNAi*$^{+/-}$ (**j**) and *TH-GAL4*$^{+/-}$; *UAS-Dmp53* (**m**) flies were fed with either 1 or 5 mM PQ alone or in combination with (0,1 mM) GA. Whole mounts of dissected brains were examined for DAergic neuron morphology and number count of neurons per brain hemisphere was recorded using a fluorescent microscopy (Axiostar plus 50) in each treatment according to ref. Bonilla-Ramirez et al. (2011). There were six clusters on the posterior side observed according to ref. Mao and Davis (2009): PPM1 (unpaired), PPM2 (paired), PPM3 (paired) (protocerebral posterior medial); PPL1, PPL2ab, PPL2c and PPL3 (paired) (protocerebral posterolateral). Average number of neurons of the medial and lateral dopaminergic neurons was scored for untreated and treated flies. The nonparametric Kruskal–Wallis test was performed to compare more than two independent groups. Differences were considered statistically significant at $P < 0.001$. The Mann–Whitney

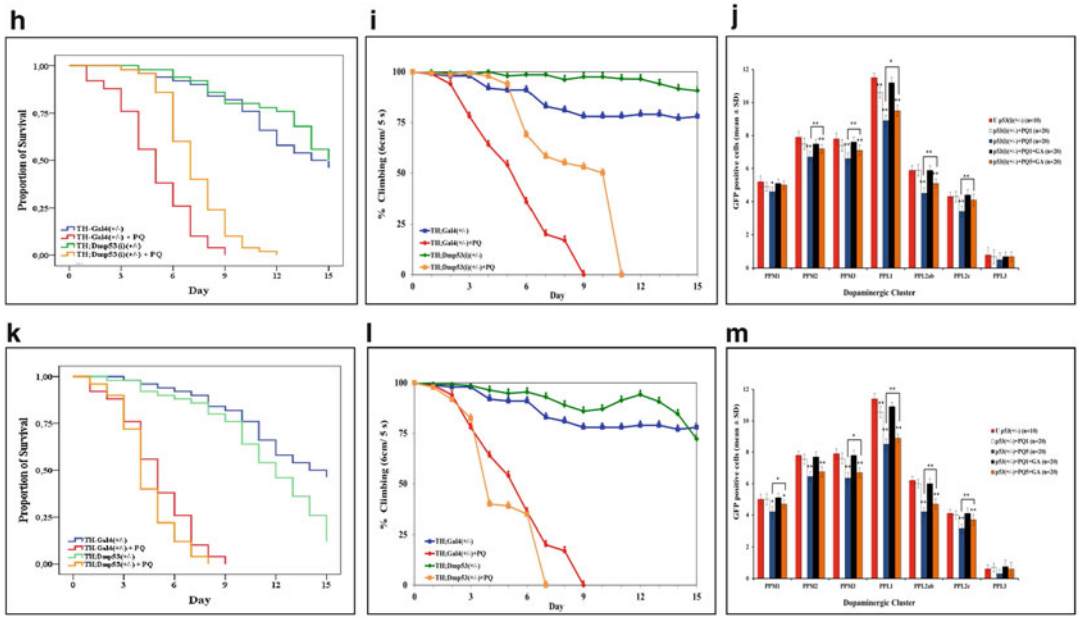


Fig. 2.4 (continued) *U* Test was used to compare differences between two independent groups. Differences were considered statistically significant at $P < 0.05$. (n=) represents the number of brain hemispheres examined (except PPM1) per treatment. Error bars indicate SEM. By day 7, PQ (1 mM) significantly eroded only PPL1 DAergic cluster in gene overexpressed/knocked down fly lines compared to untreated flies (**j**, **m**). When flies were fed with PQ (5 mM), except PPL3, all other DAergic clusters in both overexpressed/knocked down fly lines showed significant differences in the number of DAergic neurons compared with untreated flies. However, overexpressed fly lines showed a decrease in DAergic clusters compared to knocked down fly lines ($p < 0.05$). (**a**, **b**, **h** and **i** are reproduced from ref. Ortega-Arellano et al. (2013) under a Creative Commons attribution-type BY-NC)

for Aβ-neurotoxicity currently available, making it a powerful tool for gene and therapy discovery (Pandey and Nichols 2011).

2.5 *Drosophila's* Aβ Brain Is Urgently Seeking a Cure: Polyphenols Enter the Stage

Since AD is a complex and multifactorial neurodegenerative condition, an ideal therapeutic agent should have multiple targets, including, antioxidant, metal chelation, anti-aggregation, anti-inflammatory, modulate cell and neurotransmitter signaling, and ultimately have an effect on learning, memory and neurocognitive performance. Polyphenols may comply with such requeriments (e.g. Soobrattee et al. 2005; Ramassamy 2006; Weinreb et al. 2004; Rivière et al. 2007; Mandel et al. 2008; Perron and Brumaghim 2009; Kim et al. 2010; Viña et al. 2011; Sharoar et al. 2012; Hirohata et al. 2012; Kostomoiri et al. 2013; Vauzour 2014).

Polyphenols are a group of chemical substances present in plants, fruits, and vegetables, characterized by the presence of one or more than one phenol unit per molecule with several hydroxyl groups on aromatic rings. Based on differences in molecular backbone structure (Fig. 2.5) polyphenols may generally be classified into phenolic acids, flavonoids, stilbenes, and lignans. Flavonoids, the largest group of polyphenols, in turn may be classified into 2 different classes: anthocyanins and anthoxantins. This last group may be classified into flavonols, flavanones, flavones, isoflavones, and flavanols, which give origin to the catechins. In this review, we focused our attention on the effect of polyphenols as antioxidant, metal chelator, anti-amyloidogenic and anti-tau aggregator in *Drosophila*. Given that more than 8,000 polyphenolic compounds

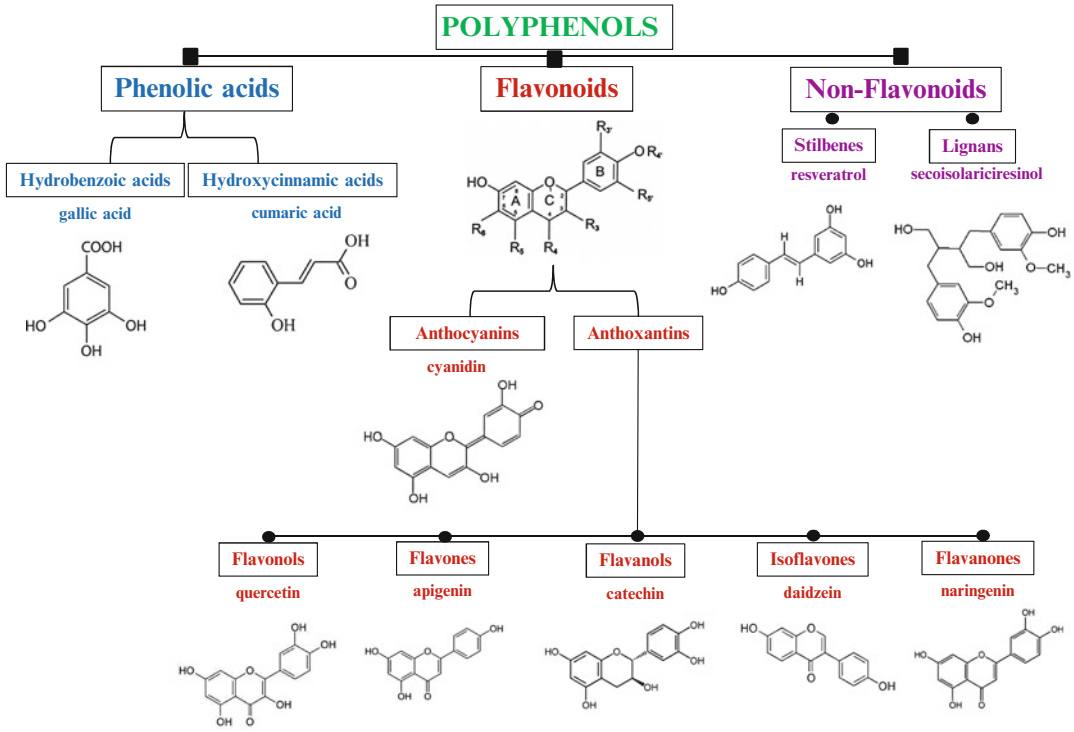


Fig. 2.5 Schematic classification of polyphenols

have been identified in various plant species, we selected a handful of known polyphenols (Fig. 2.5) to be assumed modifiers of AD. Surprisingly, no data is available to establish whether flavonoids (e.g. gallic acid, ferulic acid, caffeic acid, coumaric acid, propyl gallate, epicatechin, epigallocatechin, epigallocatechin gallate) are effective against Aβ-induced AD-like pathophysiology in *Drosophila*. This despite the fact that they are well-known to be effective as *in vitro* free radical scavengers and antioxidants (Rice-Evans et al. 1997; Sroka and Cisowski 2003; Tsimogiannis and Oreopoulou 2004, 2006; Villaño et al. 2007), *in vitro* inhibitors of Aβ-aggregation (Porat et al. 2006; Ono et al. 2012; Sato et al. 2013), *in vitro* and *in vivo* metal chelators (Hider et al. 2001; Perron et al. 2010; He et al. 2012; Bonilla-Ramirez et al. 2011), and *in vivo* gene regulatory molecules (Li et al. 2007).

Interestingly, curcumin has been shown to promote amyloid fibril conversion by reducing the pre-fibrillar/oligomeric species of Aβ, resulting in a reduced neurotoxicity in *Drosophila* (Caesar et al. 2012). Recently, it has been shown that structural characteristics, such as degrees of saturation, types of carbon skeleton and functional groups, and hydrophobicity appear to play a role in determining the inhibitory potency of curcuminoids (e.g. diarylalkyls curcumin (CCN), demethoxycurcumin (DMCCN), bisdemethoxycurcumin (BDMCCN)) on BACE-1 activity (Wang et al. 2014). Effectively, it has been found that BDMCCN has the strongest inhibitory activity toward BACE-1. Furthermore, BDMCCN (and CNN) proved not only to be effective in rescuing morphological defects observed in flies expressing APP and BACE-1 in compound eyes (GMR > APP/BACE-1), but also to improve movement in *elav-BACE-1* and *elav-APP/BACE-1* female flies. These data suggest that either curcumin (Yanagisawa et al. 2011), curcumin-derived analogs (Orlando et al. 2012) or curcuminoids from rhizomes of *Curcuma longa* might be potential pharmacological agents against AD (Mourtas et al. 2014). It is worth mentioning that ferulic acid, tannic acid and

icariin (a flavonol) have been shown to improve behavioral impairment and Alzheimer-like pathology in transgenic mice (Urano and Tohda 2010; Mori et al. 2012, 2013). We therefore expect that flavonoids, other polyphenols and non-polyphenol molecules might also be operative in transgenic AD flies (Rivière et al. 2007, 2010). This line of research is currently being undertaken in our laboratory (Bonilla-Ramirez et al. 2014).

Since ancient times, traditional medicine has been the only source of drugs to treat diseases including AD (Liu et al. 2014). Today, herbal medicines have the potential to be developed into optimal pharmaceuticals and nutraceuticals for AD because of their multi-function, multi-target characteristics (Kim and Oh 2012; Gao et al. 2013). Recently, it has been shown that a modified version of SuHeXiang Wan (SHXW), a Chinese traditional medicinal extract of 15 crude herbs, improved the developmental defects and motor activity of flies expressing Aβ42 in neurons (Hong et al. 2011). Further studies have demonstrated that inhibition of JNK or EGFR/ERK signaling pathways ameliorated the Aβ-induced lethality and locomotor deficit (Park et al. 2013). Similar to the SHXW, *Gardenia jasminoinoides Ellis* components and *Gastrodia elata blume* extract have been proved effective in improving short-term-memory in Aβ transgenic AD flies (Yu et al. 2009, 2012) and in reducing the Aβ-induced neurodegeneration in *Drosophila* (Ng et al. 2013) (for a review, see Lee et al. 2014). Concomitantly, it has been found that the *Gardenia jasminoides Ellis* fruit extracts displayed antioxidant and superoxide radical-scavenging activities, reducing power, nitrite scavenging activity, inhibition of linoleic acid oxidation activity, superoxide dismutase-like activity and catalase activity (Debnath et al. 2011). Most importantly, the antioxidant and superoxide radical-scavenging activities were highly correlated with the phenolic and polyphenol content. These findings may explain the *Gardenia* extract effect on memory in Aβ-*Drosophila* model. Another polyphenol-enriched extract, grape-seed polyphenolic extract, improves the eye phenotype in a *Drosophila* model of tauopathy (Pfleger et al. 2010). In conclusion, there is little doubt that polyphenols (Fraga et al. 2010) and/or natural products (Ansari and Khodagholi 2013) exert several beneficial effects, not least potent antioxidant activity (Galleano et al. 2010) on transgenic Aβ *Drosophila melanogaster* or PQ-exposed flies (Ortega-Arellano et al. 2011; Jimenez-Del-Rio et al. 2008, 2010).

Notwithstanding, several key questions remain unanswered. Why have antioxidant treatments largely failed in AD? (Persson et al. 2014). Can polyphenol/natural product in vitro actions be translated to benefit animal models and ultimately the human condition? Can polyphenols/natural products retard memory loss in early-onset AD *PSEN 1* E280A presymptomatic patients? Last but not least, are we dealing with an intractable neurological disorder? Further investigations are warranted to address these issues.

Acknowledgements The work was supported by "Comité para el Desarrollo de la Investigación", CODI-UdeA (Grant #EO1617 to MJimenez-Del-Rio). We thank Ortega-Arellano H for assistance with Fig. 2.4 and Table (Learning and memory, apoptosis) data, and Bonilla-Ramirez L for assistance with Table (APP, metal metabolism) data.

References

Abdelwahid E, Rolland S, Teng X, Conradt B, Hardwick JM, White K (2011) Mitochondrial involvement in cell death of non-mammalian eukaryotes. Biochim Biophys Acta 1813(4):597–607. doi:10.1016/j.bbamcr.2010.10.008

Abramov AY, Canevari L, Duchen MR (2004) Beta-amyloid peptides induce mitochondrial dysfunction and oxidative stress in astrocytes and death of neurons through activation of NADPH oxidase. J Neurosci 24(2):565–575. doi:10.1523/JNEUROSCI.4042-03.2004

Acosta-Baena N, Sepulveda-Falla D, Lopera-Gomez CM, Jaramillo-Elorza MC, Moreno S, Aguirre-Acevedo DC, Saldarriaga A, Lopera F (2011) Pre-dementia clinical stages in presenilin 1 E280A familial early-onset Alzheimer's disease: a retrospective cohort study. Lancet Neurol 10(3):213–220. doi:10.1016/S1474-4422(10)70323-9

Aisen PS (2009) Alzheimer's disease therapeutic research: the path forward. Alzheimers Res Ther 1(1):2. doi:10.1186/alzrt2

Ali YO, Escala W, Ruan K, Zhai RG (2011) Assaying locomotor, learning, and memory deficits in Drosophila

models of neurodegeneration. J Vis Exp (49). pii: 2504. doi:10.3791/2504

Aliev G, Obrenovich ME, Reddy VP, Shenk JC, Moreira PI, Nunomura A, Zhu X, Smith MA, Perry G (2008) Antioxidant therapy in Alzheimer's disease: theory and practice. Mini Rev Med Chem 8(13): 1395–1406

Alzheimer (Munich): about a peculiar disease of the cerebral cortex (2000) Paper presented at the 37th meeting of Psychiatrists of Southwestern Germany, Tubingen, 3rd and 4th Nov 1906

Alzheimer's Disease Collaborative G (1995) The structure of the presenilin 1 (S182) gene and identification of six novel mutations in early onset AD families. Nat Genet 11(2):219–222. doi:10.1038/ng1095-219

Ansari N, Khodagholi F (2013) Natural products as promising drug candidates for the treatment of Alzheimer's disease: molecular mechanism aspect. Curr Neuropharmacol 11(4):414–429. doi:10.2174/1570159X11311040005

Arcos-Burgos M, Muenke M (2002) Genetics of population isolates. Clin Genet 61(4):233–247

Ayutyanont N, Langbaum JB, Hendrix SB, Chen K, Fleisher AS, Friesenhahn M, Ward M, Aguirre C, Acosta-Baena N, Madrigal L, Muñoz C, Tirado V, Moreno S, Tariot PN, Lopera F, Reiman EM (2014) The alzheimer's prevention initiative composite cognitive test score: sample size estimates for the evaluation of preclinical alzheimer's disease treatments in presenilin 1 E280A mutation carriers. J Clin Psychiatry 75(6):652–660. doi:10.4088/JCP.13m08927

Barco A, Bailey CH, Kandel ER (2006) Common molecular mechanisms in explicit and implicit memory. J Neurochem 97(6):1520–1533. doi:10.1111/j.1471-4159.2006.03870.x

Barone E, Di Domenico F, Butterfield DA (2014) Statins more than cholesterol lowering agents in Alzheimer disease: their pleiotropic functions as potential therapeutic targets. Biochem Pharmacol 88(4):605–616. doi:10.1016/j.bcp.2013.10.030

Baulac S, Lu H, Strahle J, Yang T, Goldberg MS, Shen J, Schlossmacher MG, Lemere CA, Lu Q, Xia W (2009) Increased DJ-1 expression under oxidative stress and in Alzheimer's disease brains. Mol Neurodegener 4:12. doi:10.1186/1750-1326-4-12

Bayer TA, Wirths O (2014) Focusing the amyloid cascade hypothesis on N-truncated Abeta peptides as drug targets against Alzheimer's disease. Acta Neuropathol 127(6):787–801. doi:10.1007/s00401-014-1287-x

Beckett C, Nalivaeva NN, Belyaev ND, Turner AJ (2012) Nuclear signalling by membrane protein intracellular domains: the AICD enigma. Cell Signal 24(2):402–409. doi:10.1016/j.cellsig.2011.10.007

Behl C, Davis JB, Lesley R, Schubert D (1994) Hydrogen peroxide mediates amyloid beta protein toxicity. Cell 77(6):817–827

Berry JA, Cervantes-Sandoval I, Nicholas EP, Davis RL (2012) Dopamine is required for learning and forgetting in Drosophila. Neuron 74(3):530–542. doi:10.1016/j.neuron.2012.04.007

Bettens K, Sleegers K, Van Broeckhoven C (2010) Current status on Alzheimer disease molecular genetics: from past, to present, to future. Hum Mol Genet 19(R1):R4–R11. doi:10.1093/hmg/ddq142

Bobes MA, Garcia YF, Lopera F, Quiroz YT, Galan L, Vega M, Trujillo N, Valdes-Sosa M, Valdes-Sosa P (2010) ERP generator anomalies in presymptomatic carriers of the Alzheimer's disease E280A PS-1 mutation. Hum Brain Mapp 31(2):247–265. doi:10.1002/hbm.20861

Bonilla-Ramirez L, Jimenez-Del-Rio M, Velez-Pardo C (2011) Acute and chronic metal exposure impairs locomotion activity in Drosophila melanogaster: a model to study Parkinsonism. Biometals 24(6):1045–1057. doi:10.1007/s10534-011-9463-0

Bonilla-Ramirez L, Jimenez-Del-Rio M, Velez-Pardo C (2014) Efecto del flavanol EGCG y Curcuminoides en *Drosophila melanogaster* que expresa Ab1-42-TAU: modelo de neuroproteccion en la enfermedad de Alzheimer. [Effect of the flavanol EGCG and Curcuminoids in Drosophila melanogaster which expresses Ab1-42-TAU: neuroprotection model in Alzheimer's disease]. Paper presented at the IX Congreso Nacional de Neurociencias/X Seminario Internacional de Neurociencias, Cartagena, 15–17 May 2014

Boutajangout A, Wisniewski T (2014) Tau-based therapeutic approaches for Alzheimer's disease – a mini-review. Gerontology. doi:10.1159/000358875

Burgold S, Filser S, Dorostkar MM, Schmidt B, Herms J (2014) In vivo imaging reveals sigmoidal growth kinetic of β-amyloid plaques. Acta Neuropathol Commun 2(1):30. doi:10.1186/2051-5960-2-30

Butterfield DA, Sultana R (2011) Methionine-35 of aβ(1–42): importance for oxidative stress in Alzheimer disease. J Amino Acids 2011:198430. doi:10.4061/2011/198430

Butterfield DA, Drake J, Pocernich C, Castegna A (2001) Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid beta-peptide. Trends Mol Med 7(12):548–554

Butterfield DA, Swomley AM, Sultana R (2013) Amyloid β-peptide (1–42)-induced oxidative stress in Alzheimer disease: importance in disease pathogenesis and progression. Antioxid Redox Signal 19(8):823–835. doi:10.1089/ars.2012.5027

Caesar I, Jonson M, Nilsson KP, Thor S, Hammarström P (2012) Curcumin promotes A-beta fibrillation and reduces neurotoxicity in transgenic Drosophila. PLoS One 7(2), e31424. doi:10.1371/journal.pone.0031424

Campbell RA, Turner GC (2010) The mushroom body. Curr Biol 20(1):R11–R12. doi:10.1016/j.cub.2009.10.031

Capetillo-Zarate E, Gracia L, Tampellini D, Gouras GK (2012) Intraneuronal Aβ accumulation, amyloid plaques, and synapse pathology in Alzheimer's disease. Neurodegener Dis 10(1–4):56–59. doi:10.1159/000334762

Carmine-Simmen K, Proctor T, Tschäpe J, Poeck B, Triphan T, Strauss R, Kretzschmar D (2009) Neurotoxic effects induced by the Drosophila amyloid-beta

peptide suggest a conserved toxic function. Neurobiol Dis 33(2):274–281. doi:10.1016/j.nbd.2008.10.014

Cash DM, Ridgway GR, Liang Y, Ryan NS, Kinnunen KM, Yeatman T, Malone IB, Benzinger TL, Jack CR, Thompson PM, Ghetti BF, Saykin AJ, Masters CL, Ringman JM, Salloway SP, Schofield PR, Sperling RA, Cairns NJ, Marcus DS, Xiong C, Bateman RJ, Morris JC, Rossor MN, Ourselin S, Fox NC, (DIAN) DIAN (2013) The pattern of atrophy in familial Alzheimer disease: volumetric MRI results from the DIAN study. Neurology 81(16):1425–1433. doi:10.1212/WNL.0b013e3182a841c6

Cenini G, Sultana R, Memo M, Butterfield DA (2008) Elevated levels of pro-apoptotic p53 and its oxidative modification by the lipid peroxidation product, HNE, in brain from subjects with amnestic mild cognitive impairment and Alzheimer's disease. J Cell Mol Med 12(3): 987–994. doi:10.1111/j.1582-4934.2008.00163.x

Chabrier MA, Blurton-Jones M, Agazaryan AA, Nerhus JL, Martinez-Coria H, LaFerla FM (2012) Soluble aβ promotes wild-type tau pathology in vivo. J Neurosci 32(48):17345–17350. doi:10.1523/JNEUROSCI.0172-12.2012

Chakraborty R, Vepuri V, Mhatre SD, Paddock BE, Miller S, Michelson SJ, Delvadia R, Desai A, Vinokur M, Melicharek DJ, Utreja S, Khandelwal P, Ansaloni S, Goldstein LE, Moir RD, Lee JC, Tabb LP, Saunders AJ, Marenda DR (2011) Characterization of a Drosophila Alzheimer's disease model: pharmacological rescue of cognitive defects. PLoS One 6(6), e20799. doi:10.1371/journal.pone.0020799

Chan KY, Wang W, Wu JJ, Liu L, Theodoratou E, Car J, Middleton L, Russ TC, Deary IJ, Campbell H, Rudan I, Global Health Epidemiology Reference Group (GHERG) (2013) Epidemiology of Alzheimer's disease and other forms of dementia in China, 1990–2010: a systematic review and analysis. Lancet 381(9882):2016–2023. doi:10.1016/S0140-6736(13)60221-4

Choi J, Sullards MC, Olzmann JA, Rees HD, Weintraub ST, Bostwick DE, Gearing M, Levey AI, Chin LS, Li L (2006) Oxidative damage of DJ-1 is linked to sporadic Parkinson and Alzheimer diseases. J Biol Chem 281(16):10816–10824. doi:10.1074/jbc.M509079200

Cipriani G, Dolciotti C, Picchi L, Bonuccelli U (2011) Alzheimer and his disease: a brief history. Neurol Sci 32(2):275–279. doi:10.1007/s10072-010-0454-7

Cornejo W, Lopera F, Uribe C, Salinas M (1987) Description of a family affected by Alzheimer type presenile dementia. Acta Med Col 12:55–61

Crouch PJ, Harding SM, White AR, Camakaris J, Bush AI, Masters CL (2008) Mechanisms of A beta mediated neurodegeneration in Alzheimer's disease. Int J Biochem Cell Biol 40(2):181–198. doi:10.1016/j.biocel.2007.07.013

Crowther DC, Kinghorn KJ, Miranda E, Page R, Curry JA, Duthie FA, Gubb DC, Lomas DA (2005) Intraneuronal Abeta, non-amyloid aggregates and neurodegeneration in a Drosophila model of Alzheimer's disease. Neuroscience 132(1):123–135. doi:10.1016/j.neuroscience.2004.12.025

De Strooper B (2003) Aph-1, Pen-2, and Nicastrin with Presenilin generate an active gamma-Secretase complex. Neuron 38(1):9–12

De Strooper B, Saftig P, Craessaerts K, Vanderstichele H, Guhde G, Annaert W, Von Figura K, Van Leuven F (1998) Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. Nature 391(6665):387–390. doi:10.1038/34910

De Strooper B, Vassar R, Golde T (2010) The secretases: enzymes with therapeutic potential in Alzheimer disease. Nat Rev Neurol 6(2):99–107. doi:10.1038/nrneurol.2009.218

De Strooper B, Iwatsubo T, Wolfe MS (2012) Presenilins and γ-secretase: structure, function, and role in Alzheimer disease. Cold Spring Harb Perspect Med 2(1):a006304. doi:10.1101/cshperspect.a006304

Dean DC, Jerskey BA, Chen K, Protas H, Thiyyagura P, Roontiva A, O'Muircheartaigh J, Dirks H, Waskiewicz N, Lehman K, Siniard AL, Turk MN, Hua X, Madsen SK, Thompson PM, Fleisher AS, Huentelman MJ, Deoni SC, Reiman EM (2014) Brain differences in infants at differential genetic risk for late-onset Alzheimer disease: a cross-sectional imaging study. JAMA Neurol 71(1):11–22. doi:10.1001/jamaneurol.2013.4544

Debnath T, Park P, Deb Nath N, Samad N, Park H, Lim B (2011) Antioxidant activity of Gardenia jasminoides Ellis fruit extracts. Food Chem 128(3): 697–703

Drachman DA (2014) The amyloid hypothesis, time to move on: amyloid is the downstream result, not cause, of Alzheimer's disease. Alzheimers Dement 10(3):372–380. doi:10.1016/j.jalz.2013.11.003

Ebstein RP, Nemanov L, Lubarski G, Dano M, Trevis T, Korczyn AD (1996) Changes in expression of lymphocyte amyloid precursor protein mRNA isoforms in normal aging and Alzheimer's disease. Brain Res Mol Brain Res 35(1–2): 260–268

Elliott DA, Brand AH (2008) The GAL4 system : a versatile system for the expression of genes. Methods Mol Biol 420:79–95. doi:10.1007/978-1-59745-583-1_5

Esiri M (2001) The neuropathology of Alzheimer's disease. In: Dawbarn D, Allen S (eds) Neurobiology of Alzheimer's disease. Oxford University Press, New York, pp 33–53

Fargo K, Bleiler L (2014) Alzheimer's Association report. Alzheimers Dement 10(2):e47–e92

Fettelschoss A, Zabel F, Bachmann MF (2014) Vaccination against Alzheimer disease: an update on future strategies. Hum Vaccin Immunother 10(4)

Fleisher AS, Chen K, Quiroz YT, Jakimovich LJ, Gomez MG, Langois CM, Langbaum JB, Ayutyanont N, Roontiva A, Thiyyagura P, Lee W, Mo H, Lopez L, Moreno S, Acosta-Baena N, Giraldo M, Garcia G, Reiman RA, Huentelman MJ, Kosik

KS, Tariot PN, Lopera F, Reiman EM (2012) Florbetapir PET analysis of amyloid-β deposition in the presenilin 1 E280A autosomal dominant Alzheimer's disease kindred: a cross-sectional study. Lancet Neurol 11(12):1057–1065. doi:10.1016/S1474-4422(12)70227-2

Folwell J, Cowan CM, Ubhi KK, Shiabh H, Newman TA, Shepherd D, Mudher A (2010) Abeta exacerbates the neuronal dysfunction caused by human tau expression in a Drosophila model of Alzheimer's disease. Exp Neurol 223(2):401–409. doi:10.1016/j.expneurol.2009.09.014

Foucaud J, Burns JG, Mery F (2010) Use of spatial information and search strategies in a water maze analog in Drosophila melanogaster. PLoS One 5(12), e15231. doi:10.1371/journal.pone.0015231

Fraga CG, Galleano M, Verstraeten SV, Oteiza PI (2010) Basic biochemical mechanisms behind the health benefits of polyphenols. Mol Aspects Med 31(6):435–445. doi:10.1016/j.mam.2010.09.006

Frank DA, Greenberg ME (1994) CREB: a mediator of long-term memory from mollusks to mammals. Cell 79(1):5–8

Frautschy SA, Cole GM (2010) Why pleiotropic interventions are needed for Alzheimer's disease. Mol Neurobiol 41(2–3):392–409. doi:10.1007/s12035-010-8137-1

Fu Y, Maianu L, Melbert BR, Garvey WT (2004) Facilitative glucose transporter gene expression in human lymphocytes, monocytes, and macrophages: a role for GLUT isoforms 1, 3, and 5 in the immune response and foam cell formation. Blood Cells Mol Dis 32(1):182–190

Fuchs SY, Adler V, Pincus MR, Ronai Z (1998) MEKK1/JNK signaling stabilizes and activates p53. Proc Natl Acad Sci U S A 95(18):10541–10546

Gaeta A, Hider RC (2005) The crucial role of metal ions in neurodegeneration: the basis for a promising therapeutic strategy. Br J Pharmacol 146(8):1041–1059. doi:10.1038/sj.bjp.0706416

Galleano M, Verstraeten SV, Oteiza PI, Fraga CG (2010) Antioxidant actions of flavonoids: thermodynamic and kinetic analysis. Arch Biochem Biophys 501(1):23–30. doi:10.1016/j.abb.2010.04.005

Gao J, Inagaki Y, Li X, Kokudo N, Tang W (2013) Research progress on natural products from traditional Chinese medicine in treatment of Alzheimer's disease. Drug Discov Ther 7(2):46–57

García-Ospina GP, Jímenez-Del Río M, Lopera F, Vélez-Pardo C (2003) Neuronal DNA damage correlates with a positive detection of c-Jun, nuclear factor kB, p53 and Par-4 transcription factors in Alzheimer's disease. Rev Neurol 36(11):1004–1010

Ghosh AK, Osswald HL (2014) BACE1 (β-secretase) inhibitors for the treatment of Alzheimer's disease. Chem Soc Rev. doi:10.1039/c3cs60460h

Giacobini E, Gold G (2013) Alzheimer disease therapy – moving from amyloid-β to tau. Nat Rev Neurol 9(12):677–686. doi:10.1038/nrneurol.2013.223

Glenner GG, Wong CW (1984) Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun 120(3):885–890

Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L, Giuffra L, Haynes A, Irving N, James L (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 349(6311):704–706. doi:10.1038/349704a0

Goedert M, Hasegawa M, Jakes R, Lawler S, Cuenda A, Cohen P (1997) Phosphorylation of microtubule-associated protein tau by stress-activated protein kinases. FEBS Lett 409(1):57–62

Golde TE (2009) The therapeutic importance of understanding mechanisms of neuronal cell death in neurodegenerative disease. Mol Neurodegener 4:8. doi:10.1186/1750-1326-4-8

Golde TE, Dickson D, Hutton M (2006) Filling the gaps in the abeta cascade hypothesis of Alzheimer's disease. Curr Alzheimer Res 3(5):421–430

Golde TE, Schneider LS, Koo EH (2011) Anti-aβ therapeutics in Alzheimer's disease: the need for a paradigm shift. Neuron 69(2):203–213. doi:10.1016/j.neuron.2011.01.002

Götz J, Chen F, van Dorpe J, Nitsch RM (2001) Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils. Science 293(5534):1491–1495. doi:10.1126/science.1062097

Graeber MB, Kösel S, Grasbon-Frodl E, Möller HJ, Mehraein P (1998) Histopathology and APOE genotype of the first Alzheimer disease patient, Auguste D. Neurogenetics 1(3):223–228

Greenough MA, Camakaris J, Bush AI (2013) Metal dyshomeostasis and oxidative stress in Alzheimer's disease. Neurochem Int 62(5):540–555. doi:10.1016/j.neuint.2012.08.014

Greeve I, Kretzschmar D, Tschäpe JA, Beyn A, Brellinger C, Schweizer M, Nitsch RM, Reifegerste R (2004) Age-dependent neurodegeneration and Alzheimer-amyloid plaque formation in transgenic Drosophila. J Neurosci 24(16):3899–3906. doi:10.1523/JNEUROSCI.0283-04.2004

Grundke-Iqbal I, Iqbal K, Quinlan M, Tung YC, Zaidi MS, Wisniewski HM (1986) Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. J Biol Chem 261(13):6084–6089

Gupta S, Barrett T, Whitmarsh AJ, Cavanagh J, Sluss HK, Dérijard B, Davis RJ (1996) Selective interaction of JNK protein kinase isoforms with transcription factors. EMBO J 15(11):2760–2770

Haass C (2004) Take five – BACE and the gamma-secretase quartet conduct Alzheimer's amyloid beta-peptide generation. EMBO J 23(3):483–488. doi:10.1038/sj.emboj.7600061

Hardy J (2006) Has the amyloid cascade hypothesis for Alzheimer's disease been proved? Curr Alzheimer Res 3(1):71–73

Hardy J (2009) The amyloid hypothesis for Alzheimer's disease: a critical reappraisal. J Neurochem 110(4):1129–1134. doi:10.1111/j.1471-4159.2009.06181.x

Hardy JA, Higgins GA (1992) Alzheimer's disease: the amyloid cascade hypothesis. Science 256(5054):184–185

Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297(5580):353–356. doi:10.1126/science.1072994

He X, Park HM, Hyung SJ, DeToma AS, Kim C, Ruotolo BT, Lim MH (2012) Exploring the reactivity of flavonoid compounds with metal-associated amyloid-β species. Dalton Trans 41(21):6558–6566. doi:10.1039/c2dt12207c

Hebert LE, Weuve J, Scherr PA, Evans DA (2013) Alzheimer disease in the United States (2010–2050) estimated using the 2010 census. Neurology 80(19):1778–1783. doi:10.1212/WNL.0b013e31828726f5

Herrera-Rivero M, Soto-Cid A, Hernández ME, Aranda-Abreu GE (2013) Tau, APP, NCT and BACE1 in lymphocytes through cognitively normal ageing and neuropathology. An Acad Bras Cienc 85(4):1489–1496. doi:10.1590/0001-376520130013

Hider RC, Liu ZD, Khodr HH (2001) Metal chelation of polyphenols. Methods Enzymol 335:190–203

Hirohata M, Ono K, Takasaki J, Takahashi R, Ikeda T, Morinaga A, Yamada M (2012) Anti-amyloidogenic effects of soybean isoflavones in vitro: fluorescence spectroscopy demonstrating direct binding to Aβ monomers, oligomers and fibrils. Biochim Biophys Acta 1822(8):1316–1324. doi:10.1016/j.bbadis.2012.05.006

Hong YK, Park SH, Lee S, Hwang S, Lee MJ, Kim D, Lee JH, Han SY, Kim ST, Kim YK, Jeon S, Koo BS, Cho KS (2011) Neuroprotective effect of SuHeXiang Wan in Drosophila models of Alzheimer's disease. J Ethnopharmacol 134(3):1028–1032. doi:10.1016/j.jep.2011.02.012

Hu Y, Fortini ME (2003) Different cofactor activities in gamma-secretase assembly: evidence for a nicastrin-Aph-1 subcomplex. J Cell Biol 161(4):685–690. doi:10.1083/jcb.200304014

Huang Y, Liu F, Grundke-Iqbal I, Iqbal K, Gong CX (2005) NF-kappaB precursor, p105, and NF-kappaB inhibitor, IkappaBgamma, are both elevated in Alzheimer disease brain. Neurosci Lett 373(2):115–118. doi:10.1016/j.neulet.2004.09.074

Hubin E, van Nuland NA, Broersen K, Pauwels K (2014) Transient dynamics of Aβ contribute to toxicity in Alzheimer's disease. Cell Mol Life Sci. doi:10.1007/s00018-014-1634-z

Iijima K, Gatt A, Iijima-Ando K (2010) Tau Ser262 phosphorylation is critical for Abeta42-induced tau toxicity in a transgenic Drosophila model of Alzheimer's disease. Hum Mol Genet 19(15):2947–2957. doi:10.1093/hmg/ddq200

Iijima-Ando K, Iijima K (2010) Transgenic Drosophila models of Alzheimer's disease and tauopathies. Brain Struct Funct 214(2–3):245–262. doi:10.1007/s00429-009-0234-4

Iqbal K, Alonso A, Gong C, Khatoon S, Kudo T, Singh T, Grundke-Iqbal I (1993) Molecular pathology of Alzheimer neurofibrillary degeneration. Acta Neurobiol Exp (Wars) 53(1):325–335

Jahn TR, Kohlhoff KJ, Scott M, Tartaglia GG, Lomas DA, Dobson CM, Vendruscolo M, Crowther DC (2011) Detection of early locomotor abnormalities in a Drosophila model of Alzheimer's disease. J Neurosci Methods 197(1):186–189. doi:10.1016/j.jneumeth.2011.01.026

Jänicke RU, Sprengart ML, Wati MR, Porter AG (1998) Caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis. J Biol Chem 273(16):9357–9360

Jimenez Del Rio M, Velez-Pardo C (2006) Insulin-like growth factor-1 prevents Abeta[25–35]/(H2O2)-induced apoptosis in lymphocytes by reciprocal NF-kappaB activation and p53 inhibition via PI3K-dependent pathway. Growth Factors 24(1):67–78. doi:10.1080/08977190500361788

Jimenez-Del-Rio M, Velez-Pardo C (2004) The hydrogen peroxide and its importance in Alzheimer's and Parkinson's disease. Curr Med Chem 4:279–285

Jimenez-Del-Rio M, Velez-Pardo C (2012) The bad, the good, and the ugly about oxidative stress. Oxid Med Cell Longev 2012:163913. doi:10.1155/2012/163913

Jimenez-Del-Rio M, Daza-Restrepo A, Velez-Pardo C (2008) The cannabinoid CP55,940 prolongs survival and improves locomotor activity in Drosophila melanogaster against paraquat: implications in Parkinson's disease. Neurosci Res 61(4):404–411. doi:10.1016/j.neures.2008.04.011

Jimenez-Del-Rio M, Guzman-Martinez C, Velez-Pardo C (2010) The effects of polyphenols on survival and locomotor activity in Drosophila melanogaster exposed to iron and paraquat. Neurochem Res 35(2):227–238. doi:10.1007/s11064-009-0046-1

Jung Y, Kim H, Min SH, Rhee SG, Jeong W (2008) Dynein light chain LC8 negatively regulates NF-kappaB through the redox-dependent interaction with IkappaBalpha. J Biol Chem 283(35):23863–23871. doi:10.1074/jbc.M803072200

Kaminsky YG, Marlatt MW, Smith MA, Kosenko EA (2010) Subcellular and metabolic examination of amyloid-beta peptides in Alzheimer disease pathogenesis: evidence for Abeta(25–35). Exp Neurol 221(1):26–37. doi:10.1016/j.expneurol.2009.09.005

Kandel ER (2012) The molecular biology of memory: cAMP, PKA, CRE, CREB-1, CREB-2, and CPEB. Mol Brain 5:14. doi:10.1186/1756-6606-5-14

Kandel ER, Dudai Y, Mayford MR (2014) The molecular and systems biology of memory. Cell 157(1):163–186. doi:10.1016/j.cell.2014.03.001

Karran E, Mercken M, De Strooper B (2011) The amyloid cascade hypothesis for Alzheimer's disease: an

appraisal for the development of therapeutics. Nat Rev Drug Discov 10(9):698–712. doi:10.1038/nrd3505

Khan A, Dobson JP, Exley C (2006) Redox cycling of iron by Abeta42. Free Radic Biol Med 40(4):557–569. doi:10.1016/j.freeradbiomed.2005.09.013

Kim HG, Oh MS (2012) Herbal medicines for the prevention and treatment of Alzheimer's disease. Curr Pharm Des 18(1):57–75

Kim J, Lee HJ, Lee KW (2010) Naturally occurring phytochemicals for the prevention of Alzheimer's disease. J Neurochem 112(6):1415–1430. doi:10.1111/j.1471-4159.2009.06562.x

Kim T, Vidal GS, Djurisic M, William CM, Birnbaum ME, Garcia KC, Hyman BT, Shatz CJ (2013) Human LilrB2 is a β-amyloid receptor and its murine homolog PirB regulates synaptic plasticity in an Alzheimer's model. Science 341(6152):1399–1404. doi:10.1126/science.1242077

Kimberly WT, Esler WP, Ye W, Ostaszewski BL, Gao J, Diehl T, Selkoe DJ, Wolfe MS (2003a) Notch and the amyloid precursor protein are cleaved by similar gamma-secretase(s). Biochemistry 42(1):137–144. doi:10.1021/bi026888g

Kimberly WT, LaVoie MJ, Ostaszewski BL, Ye W, Wolfe MS, Selkoe DJ (2003b) Gamma-secretase is a membrane protein complex comprised of presenilin, nicastrin, Aph-1, and Pen-2. Proc Natl Acad Sci U S A 100(11):6382–6387. doi:10.1073/pnas.1037392100

Klünemann HH, Fronhöfer W, Wurster H, Fischer W, Ibach B, Klein HE (2002) Alzheimer's second patient: Johann F. and his family. Ann Neurol 52(4):520–523. doi:10.1002/ana.10309

Kooijman R, Willems M, De Haas CJ, Rijkers GT, Schuurmans AL, Van Buul-Offers SC, Heijnen CJ, Zegers BJ (1992) Expression of type I insulin-like growth factor receptors on human peripheral blood mononuclear cells. Endocrinology 131(5):2244–2250. doi:10.1210/endo.131.5.1425423

Korczyn AD (2008) The amyloid cascade hypothesis. Alzheimers Dement 4(3):176–178. doi:10.1016/j.jalz.2007.11.008

Kosik KS, Munoz C, Lopez L, Arcila ML, Garcia G, Madrigal L, Moreno S, Rios Romenets S, Lopez H, Gutierrez M, Langbaum JB, Cho W, Suliman S, Tariot PN, Ho C, Reiman EM, Lopera F (2015) Homozygosity of the autosomal dominant Alzheimer disease presenilin 1 E280A mutation. Neurology 84(2):206–208.doi:10.1212/WNL.0000000000001130

Kostomoiri M, Fragkouli A, Sagnou M, Skaltsounis LA, Pelecanou M, Tsilibary EC, Tzinia AK (2013) Oleuropein, an anti-oxidant polyphenol constituent of olive promotes α-secretase cleavage of the amyloid precursor protein (AβPP). Cell Mol Neurobiol 33(1):147–154. doi:10.1007/s10571-012-9880-9

Kuhn PH, Wang H, Dislich B, Colombo A, Zeitschel U, Ellwart JW, Kremmer E, Rossner S, Lichtenthaler SF (2010) ADAM10 is the physiologically relevant, constitutive alpha-secretase of the amyloid precursor protein in primary neurons. EMBO J 29(17):3020–3032. doi:10.1038/emboj.2010.167

LaFerla FM (2010) Pathways linking Abeta and tau pathologies. Biochem Soc Trans 38(4):993–995. doi:10.1042/BST0380993

LaFerla FM, Tinkle BT, Bieberich CJ, Haudenschild CC, Jay G (1995) The Alzheimer's A beta peptide induces neurodegeneration and apoptotic cell death in transgenic mice. Nat Genet 9(1):21–30. doi:10.1038/ng0195-21

Lalli MA, Cox HC, Arcila ML, Cadavid L, Moreno S, Garcia G, Madrigal L, Reiman EM, Arcos-Burgos M, Bedoya G, Brunkow ME, Glusman G, Roach JC, Hood L, Kosik KS, Lopera F (2014) Origin of the PSEN1 E280A mutation causing early-onset Alzheimer's disease. Alzheimers Dement 10(5 Suppl):S277–S283.e10. doi:10.1016/j.jalz.2013.09.005

Lambracht-Washington D, Rosenberg RN (2013) Advances in the development of vaccines for Alzheimer's disease. Discov Med 15(84):319–326

Lang M, Fan Q, Wang L, Zheng Y, Xiao G, Wang X, Wang W, Zhong Y, Zhou B (2013) Inhibition of human high-affinity copper importer Ctr1 orthologous in the nervous system of Drosophila ameliorates Aβ42-induced Alzheimer's disease-like symptoms. Neurobiol Aging 34(11):2604–2612. doi:10.1016/j.neurobiolaging.2013.05.029

Lee JH, Cheon YH, Woo RS, Song DY, Moon C, Baik TK (2012) Evidence of early involvement of apoptosis inducing factor-induced neuronal death in Alzheimer brain. Anat Cell Biol 45(1):26–37. doi:10.5115/acb.2012.45.1.26

Lee S, Bang SM, Lee JW, Cho KS (2014) Evaluation of traditional medicines for neurodegenerative diseases using Drosophila models. Evid Based Complement Alternat Med 2014:967462. doi:10.1155/2014/967462

Lemere CA, Lopera F, Kosik KS, Lendon CL, Ossa J, Saido TC, Yamaguchi H, Ruiz A, Martinez A, Madrigal L, Hincapie L, Arango JC, Anthony DC, Koo EH, Goate AM, Selkoe DJ, Arango JC (1996) The E280A presenilin 1 Alzheimer mutation produces increased A beta 42 deposition and severe cerebellar pathology. Nat Med 2(10):1146–1150

Leuner K, Schulz K, Schütt T, Pantel J, Prvulovic D, Rhein V, Savaskan E, Czech C, Eckert A, Müller WE (2012) Peripheral mitochondrial dysfunction in Alzheimer's disease: focus on lymphocytes. Mol Neurobiol 46(1):194–204. doi:10.1007/s12035-012-8300-y

Li YM, Chan HY, Huang Y, Chen ZY (2007) Green tea catechins upregulate superoxide dismutase and catalase in fruit flies. Mol Nutr Food Res 51(5):546–554. doi:10.1002/mnfr.200600238

Liao D, Miller EC, Teravskis PJ (2014) Tau acts as a mediator for Alzheimer's disease-related synaptic deficits. Eur J Neurosci 39(7):1202–1213. doi:10.1111/ejn.12504

Liu P, Kong M, Yuan S, Liu J, Wang P (2014) History and experience: a survey of traditional chinese medicine treatment for Alzheimer's disease. Evid Based Complement Alternat Med 2014:642128. doi:10.1155/2014/642128

Londono AC, Castellanos FX, Arbelaez A, Ruiz A, Aguirre-Acevedo DC, Richardson AM, Easteal S, Lidbury BA, Arcos-Burgos M, Lopera F (2013) An (1)H-MRS framework predicts the onset of Alzheimer's disease symptoms in PSEN1 mutation carriers. Alzheimers Dement. doi:10.1016/j.jalz.2013.08.282

Lopera A, Arcos M, Madrigal L, Kosik K, Cornejo W, Ossa J (1994) Alzheimer type presenile dementia with familial aggregation in Antioquia, Colombia. Acta Neurol Col 10:173–187

Lopera F, Ardilla A, Martínez A, Madrigal L, Arango-Viana JC, Lemere CA, Arango-Lasprilla JC, Hincapíe L, Arcos-Burgos M, Ossa JE, Behrens IM, Norton J, Lendon C, Goate AM, Ruiz-Linares A, Rosselli M, Kosik KS (1997) Clinical features of early-onset Alzheimer disease in a large kindred with an E280A presenilin-1 mutation. JAMA 277(10):793–799

Luo L, Tully T, White K (1992) Human amyloid precursor protein ameliorates behavioral deficit of flies deleted for Appl gene. Neuron 9(4):595–605

Mandel SA, Amit T, Kalfon L, Reznichenko L, Weinreb O, Youdim MB (2008) Cell signaling pathways and iron chelation in the neurorestorative activity of green tea polyphenols: special reference to epigallocatechin gallate (EGCG). J Alzheimers Dis 15(2):211–222

Mao Z, Davis RL (2009) Eight different types of dopaminergic neurons innervate the Drosophila mushroom body neuropil: anatomical and physiological heterogeneity. Front Neural Circuits 3:5. doi:10.3389/neuro.04.005.2009

Marinho HS, Real C, Cyrne L, Soares H, Antunes F (2014) Hydrogen peroxide sensing, signaling and regulation of transcription factors. Redox Biol 2:535–562. doi:10.1016/j.redox.2014.02.006

Markesbery WR (1997) Oxidative stress hypothesis in Alzheimer's disease. Free Radic Biol Med 23(1):134–147

Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K (1985) Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc Natl Acad Sci U S A 82(12):4245–4249

McLellan ME, Kajdasz ST, Hyman BT, Bacskai BJ (2003) In vivo imaging of reactive oxygen species specifically associated with thioflavine S-positive amyloid plaques by multiphoton microscopy. J Neurosci 23(6):2212–2217

Mhatre SD, Satyasi V, Killen M, Paddock BE, Moir RD, Saunders AJ, Marenda DR (2014) Synaptic abnormalities in a Drosophila model of Alzheimer's disease. Dis Model Mech 7(3):373–385. doi:10.1242/dmm.012104

Mirinics ZK, Calafat J, Udby L, Lovelock J, Kjeldsen L, Rothermund K, Sisodia SS, Borregaard N, Corey SJ (2002) Identification of the presenilins in hematopoietic cells with localization of presenilin 1 to neutrophil and platelet granules. Blood Cells Mol Dis 28(1):28–38

Morales I, Guzmán-Martínez L, Cerda-Troncoso C, Farías GA, Maccioni RB (2014) Neuroinflammation in the pathogenesis of Alzheimer's disease. A rational framework for the search of novel therapeutic approaches. Front Cell Neurosci 8:112. doi:10.3389/fncel.2014.00112

Mori T, Rezai-Zadeh K, Koyama N, Arendash GW, Yamaguchi H, Kakuda N, Horikoshi-Sakuraba Y, Tan J, Town T (2012) Tannic acid is a natural β-secretase inhibitor that prevents cognitive impairment and mitigates Alzheimer-like pathology in transgenic mice. J Biol Chem 287(9):6912–6927. doi:10.1074/jbc.M111.294025

Mori T, Koyama N, Guillot-Sestier MV, Tan J, Town T (2013) Ferulic acid is a nutraceutical β-secretase modulator that improves behavioral impairment and alzheimer-like pathology in transgenic mice. PLoS One 8(2), e55774. doi:10.1371/journal.pone.0055774

Mourtas S, Lazar AN, Markoutsa E, Duyckaerts C, Antimisiaris SG (2014) Multifunctional nanoliposomes with curcumin-lipid derivative and brain targeting functionality with potential applications for Alzheimer disease. Eur J Med Chem 80:175–183. doi:10.1016/j.ejmech.2014.04.050

Mucke L, Selkoe DJ (2012) Neurotoxicity of amyloid β-protein: synaptic and network dysfunction. Cold Spring Harb Perspect Med 2(7):a006338. doi:10.1101/cshperspect.a006338

Mullane K, Williams M (2013) Alzheimer's therapeutics: continued clinical failures question the validity of the amyloid hypothesis-but what lies beyond? Biochem Pharmacol 85(3):289–305. doi:10.1016/j.bcp.2012.11.014

Müller U, Winter P, Graeber MB (2013) A presenilin 1 mutation in the first case of Alzheimer's disease. Lancet Neurol 12(2):129–130. doi:10.1016/S1474-4422(12)70307-1

Ng CF, Ko CH, Koon CM, Xian JW, Leung PC, Fung KP, Chan HY, Lau CB (2013) The aqueous extract of rhizome of gastrodia elata protected drosophila and PC12 cells against beta-amyloid-induced neurotoxicity. Evid Based Complement Alternat Med 2013:516741. doi:10.1155/2013/516741

Nimmrich V, Ebert U (2009) Is Alzheimer's disease a result of presynaptic failure? Synaptic dysfunctions induced by oligomeric beta-amyloid. Rev Neurosci 20(1):1–12

Norberg E, Orrenius S, Zhivotovsky B (2010) Mitochondrial regulation of cell death: processing of apoptosis-inducing factor (AIF). Biochem Biophys Res Commun 396(1):95–100. doi:10.1016/j.bbrc.2010.02.163

Nunomura A, Perry G, Aliev G, Hirai K, Takeda A, Balraj EK, Jones PK, Ghanbari H, Wataya T, Shimohama S, Chiba S, Atwood CS, Petersen RB, Smith MA (2001) Oxidative damage is the earliest event in Alzheimer disease. J Neuropathol Exp Neurol 60(8):759–767

Oddo S, Caccamo A, Kitazawa M, Tseng BP, LaFerla FM (2003) Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. Neurobiol Aging 24(8):1063–1070

Ofstad TA, Zuker CS, Reiser MB (2011) Visual place learning in Drosophila melanogaster. Nature 474(7350):204–207. doi:10.1038/nature10131

Oleinik NV, Krupenko NI, Krupenko SA (2007) Cooperation between JNK1 and JNK2 in activation of p53 apoptotic pathway. Oncogene 26(51):7222–7230. doi:10.1038/sj.onc.1210526

Olovnikov IA, Kravchenko JE, Chumakov PM (2009) Homeostatic functions of the p53 tumor suppressor: regulation of energy metabolism and antioxidant defense. Semin Cancer Biol 19(1):32–41. doi:10.1016/j.semcancer.2008.11.005

Ono K, Li L, Takamura Y, Yoshiike Y, Zhu L, Han F, Mao X, Ikeda T, Takasaki J, Nishijo H, Takashima A, Teplow DB, Zagorski MG, Yamada M (2012) Phenolic compounds prevent amyloid β-protein oligomerization and synaptic dysfunction by site-specific binding. J Biol Chem 287(18):14631–14643. doi:10.1074/jbc.M111.325456

Oortveld MA, Keerthikumar S, Oti M, Nijhof B, Fernandes AC, Kochinke K, Castells-Nobau A, van Engelen E, Ellenkamp T, Eshuis L, Galy A, van Bokhoven H, Habermann B, Brunner HG, Zweier C, Verstreken P, Huynen MA, Schenck A (2013) Human intellectual disability genes form conserved functional modules in Drosophila. PLoS Genet 9(10), e1003911. doi:10.1371/journal.pgen.1003911

Orlando RA, Gonzales AM, Royer RE, Deck LM, Vander Jagt DL (2012) A chemical analog of curcumin as an improved inhibitor of amyloid Abeta oligomerization. PLoS One 7(3), e31869. doi:10.1371/journal.pone.0031869

Ortega-Arellano HF, Jimenez-Del-Rio M, Velez-Pardo C (2011) Life span and locomotor activity modification by glucose and polyphenols in Drosophila melanogaster chronically exposed to oxidative stress-stimuli: implications in Parkinson's disease. Neurochem Res 36(6):1073–1086. doi:10.1007/s11064-011-0451-0

Ortega-Arellano HF, Jimenez-Del-Rio M, Velez-Pardo C (2013) Dmp53, basket and drICE gene knockdown and polyphenol gallic acid increase life span and locomotor activity in a Drosophila Parkinson's disease model. Genet Mol Biol 36(4):608–615. doi:10.1590/S1415-47572013000400020

Pagani L, Eckert A (2011) Amyloid-Beta interaction with mitochondria. Int J Alzheimers Dis 2011:925050. doi:10.4061/2011/925050

Palop JJ, Mucke L (2010) Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. Nat Neurosci 13(7):812–818. doi:10.1038/nn.2583

Pandey UB, Nichols CD (2011) Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery. Pharmacol Rev 63(2):411–436. doi:10.1124/pr.110.003293

Park SH, Lee S, Hong YK, Hwang S, Lee JH, Bang SM, Kim YK, Koo BS, Lee IS, Cho KS (2013) Suppressive effects of SuHeXiang Wan on amyloid-β42-induced extracellular signal-regulated kinase hyperactivation and glial cell proliferation in a transgenic Drosophila model of Alzheimer's disease. Biol Pharm Bull 36(3):390–398

Parodi J, Sepúlveda FJ, Roa J, Opazo C, Inestrosa NC, Aguayo LG (2010) Beta-amyloid causes depletion of synaptic vesicles leading to neurotransmission failure. J Biol Chem 285(4):2506–2514. doi:10.1074/jbc.M109.030023

Perisse E, Burke C, Huetteroth W, Waddell S (2013a) Shocking revelations and saccharin sweetness in the study of Drosophila olfactory memory. Curr Biol 23(17):R752–R763. doi:10.1016/j.cub.2013.07.060

Perisse E, Yin Y, Lin AC, Lin S, Huetteroth W, Waddell S (2013b) Different kenyon cell populations drive learned approach and avoidance in Drosophila. Neuron 79(5):945–956. doi:10.1016/j.neuron.2013.07.045

Perron NR, Brumaghim JL (2009) A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. Cell Biochem Biophys 53(2):75–100. doi:10.1007/s12013-009-9043-x

Perron NR, Wang HC, Deguire SN, Jenkins M, Lawson M, Brumaghim JL (2010) Kinetics of iron oxidation upon polyphenol binding. Dalton Trans 39(41):9982–9987. doi:10.1039/c0dt00752h

Persson T, Popescu BO, Cedazo-Minguez A (2014) Oxidative stress in Alzheimer's disease: why did antioxidant therapy fail? Oxid Med Cell Longev 2014:427318. doi:10.1155/2014/427318

Pfleger CM, Wang J, Friedman L, Vittorino R, Conley LM, Ho L, Fivecoat HC, Pasinetti GM (2010) Grapeseed polyphenolic extract improves the eye phenotype in a Drosophila model of tauopathy. Int J Alzheimers Dis. doi:10.4061/2010/576357

Phelps CB, Brand AH (1998) Ectopic gene expression in Drosophila using GAL4 system. Methods 14(4):367–379. doi:10.1006/meth.1998.0592

Pitman JL, DasGupta S, Krashes MJ, Leung B, Perrat PN, Waddell S (2009) There are many ways to train a fly. Fly (Austin) 3(1):3–9

Ploia C, Antoniou X, Sclip A, Grande V, Cardinetti D, Colombo A, Canu N, Benussi L, Ghidoni R, Forloni G, Borsello T (2011) JNK plays a key role in tau hyperphosphorylation in Alzheimer's disease models. J Alzheimers Dis 26(2):315–329. doi:10.3233/JAD-2011-110320

Porat Y, Abramowitz A, Gazit E (2006) Inhibition of amyloid fibril formation by polyphenols: structural similarity and aromatic interactions as a common inhibition mechanism. Chem Biol Drug Des 67(1):27–37. doi:10.1111/j.1747-0285.2005.00318.x

Praticò D (2008) Evidence of oxidative stress in Alzheimer's disease brain and antioxidant therapy: lights and shadows. Ann N Y Acad Sci 1147:70–78. doi:10.1196/annals.1427.010

Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP (2013) The global prevalence of dementia: a systematic review and metaanalysis. Alzheimers Dement 9(1):63–75.e62. doi:10.1016/j.jalz.2012.11.007

Prüßing K, Voigt A, Schulz JB (2013) Drosophila melanogaster as a model organism for Alzheimer's disease. Mol Neurodegener 8:35. doi:10.1186/1750-1326-8-35

Quiroz YT, Stern CE, Reiman EM, Brickhouse M, Ruiz A, Sperling RA, Lopera F, Dickerson BC (2013) Cortical atrophy in presymptomatic Alzheimer's disease presenilin 1 mutation carriers. J Neurol Neurosurg Psychiatry 84(5):556–561. doi:10.1136/jnnp-2012-303299

Ramassamy C (2006) Emerging role of polyphenolic compounds in the treatment of neurodegenerative diseases: a review of their intracellular targets. Eur J Pharmacol 545(1):51–64. doi:10.1016/j.ejphar.2006.06.025

Ramirez-Bermudez J (2012) Alzheimer's disease: critical notes on the history of a medical concept. Arch Med Res 43(8):595–599. doi:10.1016/j.arcmed.2012.11.008

Ran Y, Cruz PE, Ladd TB, Fauq AH, Jung JI, Matthews J, Felsenstein KM, Golde TE (2014) γ-Secretase processing and effects of γ-secretase inhibitors and modulators on long Aβ peptides in cells. J Biol Chem 289(6):3276–3287. doi:10.1074/jbc.M113.512921

Reiman EM, Langbaum JB, Fleisher AS, Caselli RJ, Chen K, Ayutyanont N, Quiroz YT, Kosik KS, Lopera F, Tariot PN (2011) Alzheimer's prevention initiative: a plan to accelerate the evaluation of presymptomatic treatments. J Alzheimers Dis 26(Suppl 3):321–329. doi:10.3233/JAD-2011-0059

Reiman EM, Quiroz YT, Fleisher AS, Chen K, Velez-Pardo C, Jimenez-Del-Rio M, Fagan AM, Shah AR, Alvarez S, Arbelaez A, Giraldo M, Acosta-Baena N, Sperling RA, Dickerson B, Stern CE, Tirado V, Munoz C, Reiman RA, Huentelman MJ, Alexander GE, Langbaum JB, Kosik KS, Tariot PN, Lopera F (2012) Brain imaging and fluid biomarker analysis in young adults at genetic risk for autosomal dominant Alzheimer's disease in the presenilin 1 E280A kindred: a case–control study. Lancet Neurol 11(12):1048–1056

Reynolds CH, Utton MA, Gibb GM, Yates A, Anderton BH (1997) Stress-activated protein kinase/c-jun N-terminal kinase phosphorylates tau protein. J Neurochem 68(4):1736–1744

Rice-Evans C, Miller N, Paganga G (1997) Antioxidant properties of phenolic compounds. Trends Plant Sci 2(4):152–159

Rio MJ, Velez-Pardo C (2008) Paraquat induces apoptosis in human lymphocytes: protective and rescue effects of glucose, cannabinoids and insulin-like growth factor-1. Growth Factors 26(1):49–60. doi:10.1080/08977190801984205

Rival T, Page RM, Chandraratna DS, Sendall TJ, Ryder E, Liu B, Lewis H, Rosahl T, Hider R, Camargo LM, Shearman MS, Crowther DC, Lomas DA (2009) Fenton chemistry and oxidative stress mediate the toxicity of the beta-amyloid peptide in a Drosophila model of Alzheimer's disease. Eur J Neurosci 29(7):1335–1347. doi:10.1111/j.1460-9568.2009.06701.x

Rivière C, Richard T, Quentin L, Krisa S, Mérillon JM, Monti JP (2007) Inhibitory activity of stilbenes on Alzheimer's beta-amyloid fibrils in vitro. Bioorg Med Chem 15(2):1160–1167. doi:10.1016/j.bmc.2006.09.069

Rivière C, Papastamoulis Y, Fortin PY, Delchier N, Andriamanarivo S, Waffo-Teguo P, Kapche GD, Amira-Guebalia H, Delaunay JC, Mérillon JM, Richard T, Monti JP (2010) New stilbene dimers against amyloid fibril formation. Bioorg Med Chem Lett 20(11):3441–3443. doi:10.1016/j.bmcl.2009.09.074

Rooke J, Pan D, Xu T, Rubin GM (1996) KUZ, a conserved metalloprotease-disintegrin protein with two roles in Drosophila neurogenesis. Science 273(5279):1227–1231

Rosen DR, Martin-Morris L, Luo LQ, White K (1989) A Drosophila gene encoding a protein resembling the human beta-amyloid protein precursor. Proc Natl Acad Sci U S A 86(7):2478–2482

Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, Ichijo H (1998) Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. EMBO J 17(9):2596–2606. doi:10.1093/emboj/17.9.2596

Sato T, Dohmae N, Qi Y, Kakuda N, Misonou H, Mitsumori R, Maruyama H, Koo EH, Haass C, Takio K, Morishima-Kawashima M, Ishiura S, Ihara Y (2003) Potential link between amyloid beta-protein 42 and C-terminal fragment gamma 49–99 of beta-amyloid precursor protein. J Biol Chem 278(27):24294–24301. doi:10.1074/jbc.M211161200

Sato M, Murakami K, Uno M, Nakagawa Y, Katayama S, Akagi K, Masuda Y, Takegoshi K, Irie K (2013) Site-specific inhibitory mechanism for amyloid β42 aggregation by catechol-type flavonoids targeting the Lys residues. J Biol Chem 288(32):23212–23224. doi:10.1074/jbc.M113.464222

Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Liao Z, Lieberburg I, Motter R, Mutter L, Soriano F, Shopp G, Vasquez N, Vandevert C, Walker S, Wogulis M, Yednock T, Games D, Seubert P (1999) Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 400(6740):173–177. doi:10.1038/22124

Schoonbroodt S, Ferreira V, Best-Belpomme M, Boelaert JR, Legrand-Poels S, Korner M, Piette J (2000) Crucial role of the amino-terminal tyrosine residue 42 and the carboxyl-terminal PEST domain of I kappa B alpha in NF-kappa B activation by an oxidative stress. J Immunol 164(8):4292–4300

Schottyky J (1932) Uber prasenile verblodungen. Z Gesamte Neurol Psychiat 140:333–387

Selznick LA, Holtzman DM, Han BH, Gökden M, Srinivasan AN, Johnson EM, Roth KA (1999) In situ immunodetection of neuronal caspase-3 activation in Alzheimer disease. J Neuropathol Exp Neurol 58(9):1020–1026

Sepúlveda FJ, Fierro H, Fernandez E, Castillo C, Peoples RW, Opazo C, Aguayo LG (2014) Nature of the neurotoxic membrane actions of amyloid-β on hippocampal neurons in Alzheimer's disease. Neurobiol Aging 35(3):472–481. doi:10.1016/j.neurobiolaging.2013.08.035

Sepulveda-Falla D, Glatzel M, Lopera F (2012) Phenotypic profile of early-onset familial Alzheimer's

disease caused by presenilin-1 E280A mutation. J Alzheimers Dis 32(1):1–12. doi:10.3233/JAD-2012-120907

Sharma AK, Pavlova ST, Kim J, Finkelstein D, Hawco NJ, Rath NP, Mirica LM (2012) Bifunctional compounds for controlling metal-mediated aggregation of the aβ42 peptide. J Am Chem Soc 134(15):6625–6636. doi:10.1021/ja210588m

Sharoar MG, Thapa A, Shahnawaz M, Ramasamy VS, Woo ER, Shin SY, Park IS (2012) Keampferol-3-O-rhamnoside abrogates amyloid beta toxicity by modulating monomers and remodeling oligomers and fibrils to non-toxic aggregates. J Biomed Sci 19:104. doi:10.1186/1423-0127-19-104

Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, Chi H, Lin C, Li G, Holman K, Tsuda T, Mar L, Foncin JF, Bruni AC, Montesi MP, Sorbi S, Rainero I, Pinessi L, Nee L, Chumakov I, Pollen D, Brookes A, Sanseau P, Polinsky RJ, Wasco W, Da Silva HA, Haines JL, Perkicak-Vance MA, Tanzi RE, Roses AD, Fraser PE, Rommens JM, St George-Hyslop PH (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature 375(6534):754–760. doi:10.1038/375754a0

Shoji M, Iwakami N, Takeuchi S, Waragai M, Suzuki M, Kanazawa I, Lippa CF, Ono S, Okazawa H (2000) JNK activation is associated with intracellular beta-amyloid accumulation. Brain Res Mol Brain Res 85(1–2):221–233

Silva AJ, Murphy GG (1999) cAMP and memory: a seminal lesson from Drosophila and Aplysia. Brain Res Bull 50(5–6):441–442

Sitaraman D, LaFerriere H, Birman S, Zars T (2012) Serotonin is critical for rewarded olfactory short-term memory in Drosophila. J Neurogenet 26(2):238–244. doi:10.3109/01677063.2012.666298

Skoulakis EM, Grammenoudi S (2006) Dunces and da Vincis: the genetics of learning and memory in Drosophila. Cell Mol Life Sci 63(9):975–988. doi:10.1007/s00018-006-6023-9

Smith DG, Cappai R, Barnham KJ (2007) The redox chemistry of the Alzheimer's disease amyloid beta peptide. Biochim Biophys Acta 1768(8):1976–1990. doi:10.1016/j.bbamem.2007.02.002

Song J, Wang S, Tan M, Jia J (2012) G1/S checkpoint proteins in peripheral blood lymphocytes are potentially diagnostic biomarkers for Alzheimer's disease. Neurosci Lett 526(2):144–149. doi:10.1016/j.neulet.2012.08.020

Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun T (2005) Phenolics as potential antioxidant therapeutic agents: mechanism and actions. Mutat Res 579(1–2):200–213. doi:10.1016/j.mrfmmm.2005.03.023

Spuch C, Ortolano S, Navarro C (2012) New insights in the amyloid-Beta interaction with mitochondria. J Aging Res 2012:324968. doi:10.1155/2012/324968

Squitti R (2012) Metals in Alzheimer's disease: a systemic perspective. Front Biosci (Landmark Ed) 17:451–472

Sroka Z, Cisowski W (2003) Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food Chem Toxicol 41(6):753–758

Stelzmann RA, Alzheimer A, Schnitzlein HN, Murtagh FR (1995) An English translation of Alzheimer's 1907 paper, "Uber eine eigenartige Erkankung der Hirnrinde". Clin Anat 8(6):429–431. doi:10.1002/ca.980080612

Stempfle D, Kanwar R, Loewer A, Fortini ME, Merdes G (2010) In vivo reconstitution of gamma-secretase in Drosophila results in substrate specificity. Mol Cell Biol 30(13):3165–3175. doi:10.1128/MCB.00030-10

Su JH, Zhao M, Anderson AJ, Srinivasan A, Cotman CW (2001) Activated caspase-3 expression in Alzheimer's and aged control brain: correlation with Alzheimer pathology. Brain Res 898(2):350–357

Sultana R, Baglioni M, Cecchetti R, Cai J, Klein JB, Bastiani P, Ruggiero C, Mecocci P, Butterfield DA (2013) Lymphocyte mitochondria: toward identification of peripheral biomarkers in the progression of Alzheimer disease. Free Radic Biol Med 65:595–606. doi:10.1016/j.freeradbiomed.2013.08.001

Tabner BJ, El-Agnaf OM, Turnbull S, German MJ, Paleologou KE, Hayashi Y, Cooper LJ, Fullwood NJ, Allsop D (2005) Hydrogen peroxide is generated during the very early stages of aggregation of the amyloid peptides implicated in Alzheimer disease and familial British dementia. J Biol Chem 280(43):35789–35792. doi:10.1074/jbc.C500238200

Takahashi N, Kariya S, Hirano M, Ueno S (2003) Two novel spliced presenilin 2 transcripts in human lymphocyte with oxidant stress and brain. Mol Cell Biochem 252(1–2):279–283

Takahashi RH, Capetillo-Zarate E, Lin MT, Milner TA, Gouras GK (2010) Co-occurrence of Alzheimer's disease ß-amyloid and τ pathologies at synapses. Neurobiol Aging 31(7):1145–1152. doi:10.1016/j.neurobiolaging.2008.07.021

Takahashi RH, Capetillo-Zarate E, Lin MT, Milner TA, Gouras GK (2013) Accumulation of intraneuronal β-amyloid 42 peptides is associated with early changes in microtubule-associated protein 2 in neurites and synapses. PLoS One 8(1), e51965. doi:10.1371/journal.pone.0051965

Tapson VF, Boni-Schnetzler M, Pilch PF, Center DM, Berman JS (1988) Structural and functional characterization of the human T lymphocyte receptor for insulin-like growth factor I in vitro. J Clin Invest 82(3):950–957. doi:10.1172/JCI113703

Tare M, Modi RM, Nainaparampil JJ, Puli OR, Bedi S, Fernandez-Funez P, Kango-Singh M, Singh A (2011) Activation of JNK signaling mediates amyloid-ß-dependent cell death. PLoS One 6(9), e24361. doi:10.1371/journal.pone.0024361

Thakur A, Wang X, Siedlak SL, Perry G, Smith MA, Zhu X (2007) c-Jun phosphorylation in Alzheimer disease. J Neurosci Res 85(8):1668–1673. doi:10.1002/jnr.21298

Tharp WG, Sarkar IN (2013) Origins of amyloid-β. BMC Genomics 14:290. doi:10.1186/1471-2164-14-290

Thies W, Bleiler L, Association As (2013) 2013 Alzheimer's disease facts and figures. Alzheimers Dement 9(2):208–245. doi:10.1016/j.jalz.2013.02.003

Troy CM, Rabacchi SA, Xu Z, Maroney AC, Connors TJ, Shelanski ML, Greene LA (2001) beta-Amyloid-induced neuronal apoptosis requires c-Jun N-terminal kinase activation. J Neurochem 77(1):157–164

Tsimogiannis D, Oreopoulou V (2004) Free radical scavenging and antioxidant activity of 5,7,3',4'-hydroxy-substituted flavonoids. Innov Food Sci Emerging Technol 5:523–528

Tsimogiannis D, Oreopoulou V (2006) The contribution of flavonoid C-ring on the DPPH free radical scavenging efficiency. A kinetic approach for the 3',4'-hydroxy substituted members. Innov Food Sci Emerging Technol 7:140–146

Unicesi (2013) Boletin de prensa #229. Accessed 1 Aug 2014

Urano T, Tohda C (2010) Icariin improves memory impairment in Alzheimer's disease model mice (5xFAD) and attenuates amyloid β-induced neurite atrophy. Phytother Res 24(11):1658–1663. doi:10.1002/ptr.3183

van der Voet M, Nijhof B, Oortveld MA, Schenck A (2014) Drosophila models of early onset cognitive disorders and their clinical applications. Neurosci Biobehav Rev. doi:10.1016/j.neubiorev.2014.01.013

Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, Luo Y, Fisher S, Fuller J, Edenson S, Lile J, Jarosinski MA, Biere AL, Curran E, Burgess T, Louis JC, Collins F, Treanor J, Rogers G, Citron M (1999) Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science 286(5440):735–741

Vauzour D (2014) Effect of flavonoids on learning, memory and neurocognitive performance: relevance and potential implications for Alzheimer's disease pathophysiology. J Sci Food Agric 94(6):1042–1056. doi:10.1002/jsfa.6473

Velez-Pardo C, Del Rio MJ (2006) Avoidance of Abeta[(25–35)]/(H(2)O(2))-induced apoptosis in lymphocytes by the cannabinoid agonists CP55,940 and JWH-015 via receptor-independent and PI3K-dependent mechanisms: role of NF-kappaB and p53. Med Chem 2(5):471–479

Velez-Pardo C, Ospina GG, Jimenez del Rio M (2002) Abeta[25–35] peptide and iron promote apoptosis in lymphocytes by an oxidative stress mechanism: involvement of H2O2, caspase-3, NF-kappaB, p53 and c-Jun. Neurotoxicology 23(3):351–365

Velez-Pardo C, Arellano JI, Cardona-Gomez P, Jimenez Del Rio M, Lopera F, De Felipe J (2004) CA1 hippocampal neuronal loss in familial Alzheimer's disease presenilin-1 E280A mutation is related to epilepsy. Epilepsia 45(7):751–756. doi:10.1111/j.0013-9580.2004.55403.x

Villaño D, Fernández-Pachón MS, Moyá ML, Troncoso AM, García-Parrilla MC (2007) Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta 71(1):230–235. doi:10.1016/j.talanta.2006.03.050

Viña J, Lloret A, Giraldo E, Badia MC, Alonso MD (2011) Antioxidant pathways in Alzheimer's disease: possibilities of intervention. Curr Pharm Des 17(35):3861–3864

Waddell S (2010) Dopamine reveals neural circuit mechanisms of fly memory. Trends Neurosci 33(10):457–464. doi:10.1016/j.tins.2010.07.001

Wang X, Kim JR, Lee SB, Kim YJ, Jung MY, Kwon HW, Ahn YJ (2014) Effects of curcuminoids identified in rhizomes of Curcuma longa on BACE-1 inhibitory and behavioral activity and lifespan of Alzheimer's disease Drosophila models. BMC Complement Altern Med 14:88. doi:10.1186/1472-6882-14-88

Weinreb O, Mandel S, Amit T, Youdim MB (2004) Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases. J Nutr Biochem 15(9):506–516. doi:10.1016/j.jnutbio.2004.05.002

Weuve J, Hebert LE, Scherr PA, Evans DA (2014) Deaths in the United States among persons with Alzheimer's disease (2010–2050). Alzheimers Dement 10(2):e40–e46. doi:10.1016/j.jalz.2014.01.004

Wolfe MS, Xia W, Ostaszewski BL, Diehl TS, Kimberly WT, Selkoe DJ (1999) Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. Nature 398(6727):513–517. doi:10.1038/19077

Yanagisawa D, Taguchi H, Yamamoto A, Shirai N, Hirao K, Tooyama I (2011) Curcuminoid binds to amyloid-β1-42 oligomer and fibril. J Alzheimers Dis 24(Suppl 2):33–42. doi:10.3233/JAD-2011-102100

Yang D, Tournier C, Wysk M, Lu HT, Xu J, Davis RJ, Flavell RA (1997) Targeted disruption of the MKK4 gene causes embryonic death, inhibition of c-Jun NH2-terminal kinase activation, and defects in AP-1 transcriptional activity. Proc Natl Acad Sci U S A 94(7):3004–3009

Yu Y, Xie ZL, Gao H, Ma WW, Dai Y, Wang Y, Zhong Y, Yao XS (2009) Bioactive iridoid glucosides from the fruit of Gardenia jasminoides. J Nat Prod 72(8):1459–1464. doi:10.1021/np900176q

Yu W, Mechawar N, Krantic S, Quirion R (2010) Evidence for the involvement of apoptosis-inducing factor-mediated caspase-independent neuronal death in Alzheimer disease. Am J Pathol 176(5):2209–2218. doi:10.2353/ajpath.2010.090496

Yu Y, Feng XL, Gao H, Xie ZL, Dai Y, Huang XJ, Kurihara H, Ye WC, Zhong Y, Yao XS (2012) Chemical constituents from the fruits of Gardenia jasminoides Ellis. Fitoterapia 83(3):563–567. doi:10.1016/j.fitote.2011.12.027

Zhao M, Su J, Head E, Cotman CW (2003) Accumulation of caspase cleaved amyloid precursor protein represents an early neurodegenerative event in aging and in Alzheimer's disease. Neurobiol Dis 14(3):391–403

Zou H, Li Y, Liu X, Wang X (1999) An APAF-1.cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9. J Biol Chem 274(17):11549–11556

Biflavonoids as Potential Small Molecule Therapeutics for Alzheimer's Disease

3

Arjun Thapa and Eva Y. Chi

Abstract

Flavonoids are naturally occurring phytochemicals found in a variety of fruits and vegetables and offer color, flavor, aroma, nutritional and health benefits. Flavonoids have been found to play a neuroprotective role by inhibiting and/or modifying the self-assembly of the amyloid-β (Aβ) peptide into oligomers and fibrils, which are linked to the pathogenesis of Alzheimer's disease. The neuroprotective efficacy of flavonoids has been found to strongly depend on their structure and functional groups. Flavonoids may exist in monomeric, as well as di-, tri-, tetra- or polymeric form through C-C or C-O-C linkages. It has been shown that flavonoids containing two or more units, e.g., biflavonoids, exert greater biological activity than their respective monoflavonoids. For instance, biflavonoids have the ability to distinctly alter Aβ aggregation and more effectively reduce the toxicity of Aβ oligomers compared to the monoflavonoid moieties. Although the molecular mechanisms remain to be elucidated, flavonoids have been shown to alter the Aβ aggregation pathway to yield non-toxic, unstructured Aβ aggregates, as well as directly exerting a neuroprotective effect to cells. In this chapter, we review biflavonoid-mediated Aβ aggregation and toxicity, and highlight the beneficial roles biflavonoids can potentially play in the prevention and treatment of Alzheimer's disease.

Keywords

Alzheimer's disease • Amyloid-β peptide (Aβ) • Protein aggregation • Neurotoxicity • Polyphenols • Biflavonoids

3.1 Alzheimer's Disease

Alzheimer's disease (AD) is the most common neurodegenerative disorder that causes memory loss and cognitive impairment, especially among

A. Thapa • E.Y. Chi, Ph.D. (✉)
Department of Chemical and Biological Engineering and the Center for Biomedical Engineering, University of New Mexico, Albuquerque, New Mexico
e-mail: evachi@unm.edu

N. Vassallo (ed.), *Natural Compounds as Therapeutic Agents for Amyloidogenic Diseases*, Advances in Experimental Medicine and Biology 863, DOI 10.1007/978-3-319-18365-7_3

the elderly (Selkoe 1991; Hardy and Selkoe 2002; Blennow et al. 2006; Pimplikar 2009). AD is chronic and complex. It is the fourth leading cause of death and accounts for 50–80 % of all dementia cases. Nearly 10 % of individuals above 65 years of age are afflicted with AD and it is believed that about 30 million people world wide are living with this devastating illness (Hamley 2012; Thies and Bleiler 2013). Genetic mutations in three genes that encode the amyloid precursor protein (*APP*), presenilin-1 (*PSEN-1*), and presenilin-2 (*PSEN-2*) proteins, as well as polymorphisms in apolipoprotein E (*APOE*) have been shown to be linked to early-onset (or familial) AD (Hamley 2012; Blennow et al. 2006; Yu et al. 2014). Close to 25 other genes have also been documented and recognized to increase the risk for AD. In addition to genetics, aging, environmental factors, diet that promotes obesity, and an inactive life style are also known risk factors for late-onset (or sporadic) AD, which accounts for over 95 % of all AD cases (Hamley 2012; Blennow et al. 2006). The mechanisms by which these risk factors contribute to AD pathogenesis, however, are largely unknown. In fact, many aspects of AD etiology and disease progression remain unclear. Accumulating evidence indicate that the misfolding and aggregation of the amyloid-β (Aβ) peptide, which is a fragment of the APP protein, is a central pathogenic event associated with AD development (Suh 1997; Hardy and Selkoe 2002; Haass et al. 1993). Several reports have also shown that reactive oxygen and nitrogen species, metal ions, and inflammation can be triggers for AD (Nuzzo et al. 2014; Cuajungco et al. 2000; Chauhan and Chauhan 2006; Gu et al. 2008).

The Aβ peptides are generated from the abnormal processing of APP by α-, β- and γ- secretase enzymes (Hamley 2012; Suh 1997; Haass et al. 1993). Although the biological function of APP is still unclear, the protein has been found to be involved in multiple cellular processes, including neuronal growth and survival (Nie et al. 2011; Haass et al. 1993). APP cleavage by β-secretase at methionine 671 position produces a soluble APP fragment and a C-terminal fragment with 99 amino acids. The C-terminal fragment is further cleaved by γ-secretase at valine 711 and alanine 713 positions to generate Aβ isoforms of 40 or 42 amino acid residues (Aβ40 or Aβ42) depending upon the cleavage site. These two Aβ isoforms are the major disease-causing peptides (Suh 1997; Hamley 2012; Blennow et al. 2006). Alternatively, α-secretase cleaves APP at lysine 687 and generates a soluble APP fragment and a C-terminal fragment of 83 amino acid residues. The latter fragment is further cleaved by γ-secretase to produce an Aβ (17–42) fragment, which is known as P3 (Suh 1997; Blennow et al. 2006). The P3 fragment does not undergo spontaneous aggregation like the other Aβ40 or Aβ42 peptides generated by the sequential cleavage of β- and γ- secretases (Suh 1997; Hamley 2012; Blennow et al. 2006). Moreover, the soluble fragment has been described to be neurotrophic and neuroprotective (Nie et al. 2011).

Genetic mutations that affect APP, as in familial AD, have been shown to increase the protein's susceptibility to be cleaved by secretases, resulting in higher rates of Aβ production. The increased Aβ production and subsequent aggregation and accumulation of the peptides into insoluble deposits (or amyloid plaques) over time is believed to cause neurodegeneration that leads to AD (Nie et al. 2011; Hamley 2012; Haass et al. 1993). It is important to note that no mutations in APP are found in sporadic AD, which represents over 95 % of all AD cases, that enhances Aβ production (Hamley 2012; Nie et al. 2011). Regardless of mutation-associated variations in APP processing and Aβ peptide production, the Aβ peptides can self-associate following their release from APP and over time gain pathogenicity. Non-genetic factors that influence AD development include age, oxidative stress, inflammation, and increased levels of metal ions. In fact, aging is recognized as the primary risk factor. Reactive oxygen species (ROS) and metal ions accumulated during aging greatly influence AD progression. ROS has been shown to induce Aβ peptide misfolding and aggregation and enhance Aβ toxicity (Gu et al. 2008; Chauhan and Chauhan 2006; Cuajungco et al. 2000). Accumulating knowledge

suggests that the aggregation and accumulation of Aβ, as well as the factors that influence Aβ aggregation, contribute toward AD pathogenesis.

The progressive self-association and deposition of Aβ peptides that leads to amyloid plaque formation comprise a histopathological hallmark in AD brains (Hardy and Selkoe 2002; Roychaudhuri et al. 2009; Blennow et al. 2006). It is also generally believed that the aggregation process itself is directly involved in the development of AD. However, the mechanism by which the aggregation process occurs and the pathway by which the peptide aggregates cause widespread neuronal death in the brain are still under investigation. Aggregation of Aβ has been shown to trigger oxidative stress, inflammation, and neurotoxicity resulting in cognitive dysfunction (Hardy and Selkoe 2002). Currently available therapeutics for AD provide moderate and temporary relief of symptoms (Blennow et al. 2006). A significant number of clinical trials for AD have been unsuccessful. The lack of a clear understanding of the molecular mechanisms of Aβ misfolding and aggregation is one of the major challenges and an obstacle in AD drug discovery (Uversky 2010; Hardy and Selkoe 2002).

3.2 Aβ-Peptide Toxicity in Alzheimer's Disease

It is now well accepted that Aβ peptide production and subsequent aggregation is a major causative factor for AD pathogenesis (Hardy and Selkoe 2002; Pike et al. 1991; Roychaudhuri et al. 2009). Although various N- or C-terminus truncated Aβ peptide fragments have been found in the brains of AD patients, Aβ40 and Aβ42 are the major isoforms present (Roychaudhuri et al. 2009; Suh 1997). The most abundant isoform of the Aβ peptide found in AD patients is Aβ40, which is less toxic and forms fibrils more slowly compared to Aβ42. The presence of the two additional amino acids in Aβ42, isoleucine and alanine, increases the peptide's aggregation propensity and neuronal toxicity. The production of Aβ42 has been found to be enhanced by genetic mutations linked to the familial form of AD (Findeis 2007; Blennow et al. 2006).

The native Aβ peptides are unstructured monomers with a molecular mass of 4 kDa. Aβ peptide is rich in hydrophobic amino acid residues and is amphiphilic. The C-terminus and particularly the central part of the peptide contain highly hydrophobic residues, whereas the N-terminus contains hydrophilic and charged residues. More than half of the amino acid residues in Aβ peptides are hydrophobic and are prone to aggregation (Walsh et al. 1999; Dasilva et al. 2010). Aβ peptides spontaneously undergo conformational changes from unstructured monomers to form misfolded intermediates. The misfolded Aβ species are highly unstable and undergo further aggregation to form higher-order oligomers, protofibrils, and fibrils that are rich in β-sheet structures (Walsh et al. 1999; Dasilva et al. 2010). Aβ fibrils consist of 5–6 strands of protofibrils bundled in parallel. The aggregation of Aβ peptides is fundamentally associated with its neurotoxic effects and has been found to trigger oxidative stress and inflammation (Walsh et al. 1999; Chauhan and Chauhan 2006; Pimplikar 2009; Nuzzo et al. 2014). *In vitro* and *in vivo* studies have shown that soluble globular Aβ oligomers consisting of 3 to 24 monomers (Aβ derived diffusion ligand (ADDL)) and curvilinear, beaded oligomers measuring 3–6 nm in diameter and 200 nm in length (protofibrils) formed during the early phases of the Aβ aggregation process are the potentially toxic Aβ species, rather than unstructured monomers or the aggregated end-product, fibrils (Walsh et al. 1999; Klein 2002). In fact, the Aβ aggregation process is a highly complex and heterogeneous one (Uversky 2010). Distinctly different aggregation behaviors of the two Aβ isoforms have been found (Chen and Glabe 2006). Although the structures of Aβ aggregates and mechanism of Aβ aggregation remain unclear, the steric packing of the hydrophobic residues and/or π-stacking of aromatic amino acid side chains are believed to be the underlying driving forces for Aβ aggregation (Armstrong et al. 2011; Gazit 2002).

3.3 Therapeutic Strategies for Alzheimer's Disease

As discussed above, increasing evidence suggests that Aβ aggregation is a primary cause of AD pathogenesis (Walsh et al. 1999; Chromy et al. 2003). Additionally, excessive generation of reactive oxygen and nitrogen species and inflammation are found in the AD brain (Gu et al. 2008; Butterfield et al. 2001). Therapeutic targets for AD remain elusive. A number of clinical trials for AD have been launched over the years and so far, results have been discouraging. It is clear that a lack of understanding of the molecular mechanisms of AD, in particular, a poor understanding of Aβ aggregation, is a major obstacle in AD drug development. There is also a lack of suitable animal models that recapitulate all of the pathological events seen in human AD brains, as well as a lack of biomarkers to detect and monitor the progression of AD (Ghosh et al. 2012; Jay et al. 2011; LaFerla and Green 2012). However, significant progress has been made in advancing our understanding of AD mechanisms. *In vitro* and *in vivo* studies have indicated that the excessive generation of Aβ peptides and subsequent Aβ aggregation primarily induce Aβ neurotoxicity. Several studies indicate that lowering Aβ production and stabilizing its native form, inhibiting Aβ aggregation, and clearing Aβ pool are promising therapeutic strategies for AD (Soto et al. 2000; Blennow et al. 2006; Permanne et al. 2002). Drugs that can alleviate oxidative stress and inflammation are also potential therapeutics for AD. Below, we briefly summarize the range of therapeutic strategies that have been investigated.

3.3.1 α-, β-, and γ- Secretase Modulators

Generation of Aβ peptides is at the beginning of a cascade that leads to AD. α-, β-, and γ-secretases are aspartic acid proteases that preferentially cleave APP (Haass et al. 1993; Ghosh et al. 2012). As discussed previously, β- and γ-secretases are the principal players involved in Aβ peptide production, while α-secretase cleavage of APP has been found to prevent Aβ deposition. Inhibitors that target β- and γ-secretases as well as activators of α-secretase have therefore been considered promising candidates for treating AD.

The amyloidogenic processing of APP is initiated by β-secretase cleavage, which results in the release of a large soluble fragment and a membrane-tethered C-terminal fragment (Haass et al. 1993; Hardy and Selkoe 2002). The second cleavage in the production of Aβ peptide is mediated by γ-secretase, which cuts the membrane fragment within the transmembrane domain to release Aβ into the extracellular milieu (Pimplikar 2009; Hardy and Selkoe 2002). The cleavage mechanism and inhibition pathways of β-secretase that can reduce the rate of Aβ production are largely known. A study that characterized the structure and catalytic properties of β-secretase suggests that a large inhibitor molecule is required to fit into the relatively large-sized active site of the enzyme (Nie et al. 2011; Ghosh et al. 2012). For drug development, small molecule therapeutics with high molecular weights are expected to have poor blood-brain barrier (BBB) permeability, thus making β-secretase inhibitors generally unsuitable as drugs. The orally available drug LY2886721 has been shown to inhibit β-secretase activity, reduce brain Aβ production, and rescue the cognitive decline in transgenic AD mice (Lahiri et al. 2014; Nie et al. 2011). Unfortunately, the drug failed phase II clinical trials due to liver dysfunctions in patients (Lahiri et al. 2014).

γ-secretase is the second enzyme involved in the amyloidogenic processing of APP to generate Aβ peptides. The enzyme is a membrane-embedded protease complex that cleaves the transmembrane region of APP at multiple sites due to its heterogeneous site preference. This second cleavage of APP generates Aβ peptides of variable length (38–43 amino acids long). As discussed previously, the Aβ42 isoform is prone to oligomerization, aggregation, and fibril formation, all of which are considered critical in the development of AD pathology. A number of peptides and small molecules inhibitors of the

γ-secretase complex that block the active site or interact with the initial substrate docking site have been studied (Ghosh et al. 2012; Nie et al. 2011). The γ-secretase inhibitor semagacestat was discovered to reduce Aβ production in cell culture and also *in vivo*. However, the inhibitor was not successful in a phase III clinical trial (Nie et al. 2011; Doody et al. 2013). Semagacestat was effective at reducing Aβ peptide levels in the plasma but not in the cerebrospinal fluid. Also, the compound did not improve cognitive functions in patients (Doody et al. 2013). γ-secretase has several physiological substrates, including the Notch protein. Because Notch proteins perform a variety of cellular functions, the inhibition of γ-secretase activity may also lead to other deleterious physiological consequences (Nie et al. 2011; Ghosh et al. 2012; Blennow et al. 2006). Recent studies have shown that small molecules that modulate β- and γ-secretase activities and selectively alter Aβ production without altering signal transduction may be a suitable strategy for AD intervention (Ghosh et al. 2012; Nie et al. 2011).

In contrast to the amylodogenic processing of APP by β- and γ- secretases that generate Aβ peptides, the non-amyloidogenic processing of APP is initiated by α-secretase, which cuts APP 16 amino acids downstream of the β-secretase cleavage site. The cleavage releases a soluble fragment and a truncated transmembrane fragment that is then cleaved by γ-secretase. The truncated Aβ peptides produced are non-amyloidogenic and have been shown to be neurotrophic and neuroprotective. Thus, stimulating α-secretase activity appears to be a direct gain-of-function strategy and may be a safe route to prevent AD. However, there is also evidence that α-secretase cleaves other membrane proteins aside from APP (Nie et al. 2011; Blennow et al. 2006).

It is clear that modulating α-, β-, and γ-secretase activities may be a promising strategy for preventing and treating AD. However, efforts so far in finding β- and γ- secretase inhibitors and α-secretase activators have not been successful. A more detailed understanding of the underlying mechanisms and the complex interplay with other biochemical processes are needed for identifying novel and specific inhibitors or activators necessary for AD therapeutic development.

3.3.2 Immunotherapy

The accumulation of Aβ peptides in the brain is dependent upon a balance between its deposition (production and aggregation) and degradation (phagocytosis and proteasomal degradation) (Blennow et al. 2006; Hardy and Selkoe 2002). Antibodies that target Aβ peptides and activate the immune system can result in the induction or suppression of immune responses that enhance Aβ clearance. The injection of Aβ peptides as antigens (active immunization) or the injection of preformed antibodies against Aβ (passive immunization) have been considered as viable therapeutic strategies for treating AD (Delrieu et al. 2012; Blennow et al. 2006). Antibodies can clear Aβ by binding to the peptide in the plasma and sequestering them or by binding to Aβ in the brain for phagocytosis. Immunization with Aβ or Aβ antibodies have been shown to successfully reduce AD pathology and improve cognitive functions in AD mouse model (Rasool et al. 2013). However, although the active immunization with a mixture of Aβ42 and the adjuvant QS-21 (AN1792 Aβ vaccine) has been shown to be effective at clearing amyloid senile plaques in the human brain, the immunization did not improve cognition. Rather, AN1792 was found to induce autoimmune encephalitis (Nie et al. 2011; Delrieu et al. 2012). The failure of phase II clinical trials due to the development of meningoencephalitis in patients has raised serious concerns about the therapeutic strategy of active immunization for the treatment of AD.

3.3.3 Anti-oxidative Therapy

A number of studies have shown that the AD brain shows increased oxidative stress (Butterfield et al. 2001; Chauhan and Chauhan 2006). Reactive oxygen species (e.g., superoxide anions, hydrogen peroxides, hydroxyl and peroxyl radicals, and singlet oxygen) and reactive ni-

trogen species (e.g., nitric oxide) produced during oxidative stress are capable of damaging proteins, DNA, and lipids that subsequently result in neuronal death. A number of factors can cause neurons to be particularly vulnerable to free radical attacks. First, oxygen is abundant in the brain as the brain requires a large amount of oxygen for metabolic activities. Second, the neuronal membrane consists of high proportions of easily oxidizable polyunsaturated fatty acids. Third, neurons contain low levels of the natural anti-oxidant glutathione. Fourth, increased levels of metal ions are found in the AD brain that can potently generate free radicals and enhance protein and DNA oxidation. Moreover, the presence of heme oxygenase-1 and superoxide dismutase-1 (anti-oxidative enzymes) in senile plaques highlight the possible role of oxidative stress in AD (Chauhan and Chauhan 2006; Butterfield et al. 2001; Zhou et al. 2014). The fact that aging is a key risk factor for AD and that reactive oxygen species accumulate over time provides additional support for the free radical hypothesis of AD pathogenesis. Therefore, antioxidants taken as supplements, such as vitamin E, vitamin C, coenzyme Q10, and β-carotene, have been tested to prevent and treat AD. However, the supplements have been found to be ineffective at reducing AD symptoms (Yang et al. 2010). Some studies have shown that Aβ peptides can induce the formation of free radicals while others have shown that Aβ peptides may serve as antioxidants and compensate for the increased oxidative stress in cells. Tissue injury by itself can generate reactive oxygen species. Thus, although there is increasing evidence that oxidative stress is involved in AD, the underlying link between the two is still not properly understood.

3.3.4 Anti-inflammatory Therapy

Aside from the two major known neuropathological AD hallmarks, senile plaques and neurofibrillary tangles, a number of pathological abnormalities, including microglial activation and inflammatory processes, are found in AD brains (Nuzzo et al. 2014; Zhou et al. 2014; Kim et al. 2008). Chronic inflammatory reactions are also common symptoms among age-related neurodegenerative disorders. Inflammation is a natural immune response linked to the excessive activation of the glial cells during fighting against harmful substances or injuries. An abundant number of activated glial cells such as microglia and astrocytes are characteristically found around neurons and amyloid plaques. Inflammation mediator molecules such as cytokines, chemokines, macrophages, prostaglandins, coagulation factors, and reactive oxygen species have been detected in the AD brain (Kim et al. 2008). Genetic studies have shown that polymorphisms in some genes that encode for inflammatory interleukins 1α and 1β enhance the risk of AD (Kim et al. 2008; Nuzzo et al. 2014). These findings suggest chronic inflammation may play an important role in AD pathogenesis. However, anti-inflammatory drugs (NSAIDs) such as naproxen and celecoxib have been shown to be ineffective in reducing AD pathology and symptoms (Lyketsos et al. 2007). Recent evidence suggests that NSAIDs may lower Aβ load by inhibiting the small GTP binding protein Rho and its effector, Rho-associated kinase (Rock), instead (Gasparini et al. 2004; Blennow et al. 2006).

3.3.5 Metal Chelators

Studies have shown that APP and Aβ peptides can interact with and bind to heavy metal ions, including copper, iron, aluminum, and zinc ions. The binding can subsequently induce the misfolding and aggregation of Aβ peptides (Hane et al. 2013; Cuajungco et al. 2000). In fact, elevated levels of such metal ions have been found in the brains of AD patients, as well as in elderly individuals (Cuajungco et al. 2000). Monomeric and oligomeric Aβ have been shown to bind to metal ions, which give rise to ordered Aβ structures. Therefore, metal chelation has been explored as one of the therapeutic strategies for reducing Aβ-metal ion interactions by removing metal ions in the AD brains. Clioquinol, a copper-zinc chelator, is one of the FDA approved metal chelators for

AD (Schimmer et al. 2012). However, its use has been discontinued due to its adverse effects, including neurotoxic effects in some patients. It remains to be understood which form, in terms of conformational and aggregation state, the Aβ peptide adopts after the metal ions are removed from Aβ aggregates. Moreover, the delivery of metal chelating compounds to the Aβ enriched areas of the brain remains a challenging task (Blennow et al. 2006; Nie et al. 2011).

3.3.6 Inhibitors and Modulators of Neurotransmitters

Neurotransmitters (neurochemical messengers such as acetylcholine) are signaling molecules involved in several aspects of normal brain functioning including cognition, memory, and learning. The dysregulation of the biosynthesis of acetylcholine neurotransmitter has been found in AD (Brogi et al. 2014; Rosini et al. 2014). Anti-acetylcholinesterases, which inhibit the action of the acetylcholinesterase enzyme that breaks down acetylcholine and increase the level, duration, and action of acetylcholine, have been suggested to improve learning and memory. Examples of these inhibitors include galantamine, reminyl, razadyne, rivastigmine, and donepezil (Hamley 2012; Blennow et al. 2006). Anti-acetylcholinesterases have also been found to protect cells from free radical toxicity and Aβ-induced toxicity. In fact, cholinesterase inhibitors have been used as a first line medication for AD as they have been shown to be effective at delaying cognitive impairment. Elevated levels of another neurotransmitter, glutamate, that activates *N*-methyl-D-aspartate (NMDA) receptors and mediate excitatory signals, have also been found in AD (Johnston et al. 2006). Additionally, high concentrations of the neurotransmitter γ-aminobutyric acid (GABA), which mediates inhibitory signaling in the reactive astrocytes of the dentate gyrus, have been found. A correlation between high concentrations of GABA and deteriorations in learning and memory capacity has been shown (Wu et al. 2014). Memantine is one of the available AD prophylactic medications that block NMDA receptors. Like other inhibitors that reduce AD symptoms, the inhibitors or modulators of neurotransmitter release are not free from side effects (Blennow et al. 2006). Dizziness, headaches, nausea, vomiting, diarrhea, loss of appetite, and weight are some of the common side effects.

3.3.7 Aβ-Based Peptide Inhibitors

Given that Aβ aggregation is critical in AD development, the inhibition of Aβ aggregation is another AD therapeutic strategy that has been explored (Estrada and Soto 2007; Funke and Willbold 2012). Mutational and computational studies have revealed that residues in the hydrophobic C-terminal half of Aβ, leucine, isoleucine, phenylalanine, and aromatic amino acids, are critical for β-sheet formation in Aβ aggregates (Gazit 2002; Porat et al. 2006). Moreover, not only do the full-length Aβ peptides aggregate, truncated fragments containing the hydrophobic segment can also self-associate. Hence, Aβ peptides segments that can bind to the hydrophobic region of the full-length Aβ peptide and prevent Aβ aggregation have been considered and tested as alternative therapeutics to prevent AD (Estrada and Soto 2007; Mason et al. 2003). Specifically, a pentameric core segment of Aβ, Aβ(16–20) with the amino acid sequence of KLVFF, has been tested as a drug lead because it is able to bind to the full-length Aβ and prevent the peptide's assembly into fibrils (Pallitto et al. 1999). Soto and coworkers introduced a proline-containing Aβ(17–21) segment, which has an amino acid sequence of LVFFA, and showed that the segment reduced the propensity of full length Aβ peptides to form β-sheets (Poduslo et al. 1999; Estrada and Soto 2007). The peptide fragment LVPFF has also been found to inhibit Aβ aggregation and disrupt preformed fibrils (Mason et al. 2003). These β-sheet breaker peptides are believed to be small and hydrophobic enough to cross the BBB. Based on the Aβ(15–25) sequence, Murphy and co-workers designed a novel peptide series with a 'recognition element' homologous to Aβ and a

'disrupting element' fused to the C terminus of the peptide (Pallitto et al. 1999). It was shown that a minimum of three lysines is required to disrupt Aβ aggregation. The peptide KLVFFKKKK reduced Aβ toxicity by accelerating Aβ aggregation kinetics and altering fibril morphology. The study thus demonstrated that Aβ aggregation does not need be blocked in order to prevent Aβ toxicity. Compounds that increase the aggregation kinetics to form Aβ fibrils at faster rates can be protective as Aβ fibrils are less toxic than Aβ oligomers (Blennow et al. 2006). However, proteolysis and short *in vivo* half-life of peptide are the major obstacles associated with this class of Aβ aggregation and toxicity inhibitors.

3.3.8 Aromatic Small Molecules

Based on the observation that aromatic amino acids are abundant in aggregation-prone proteins and peptides, including Aβ peptides, Gazit and co-workers proposed that the π-π stacking of the aromatic amino acid side chains stabilizes the fibrillar conformation of Aβ and is a driving force for amyloid fibril formation (Armstrong et al. 2011; Reinke and Gestwicki 2007; Gazit 2002). In their study, a series of synthetic as well as naturally occurring compounds, including curcumin, resveratrol, apigenin, apomorphine and Congo Red, that are capable of disrupting π-π interactions were tested for their ability to inhibit Aβ fibril formation and toxicity (Gazit 2002; Porat et al. 2006). Results from the study show that compounds containing at least two aromatic structures (phenyl rings) and three hydroxyl groups linked with two to six atom linkers were effective anti-amyloid compounds (Porat et al. 2006). Two other studies have also shown that the numbers of phenyl rings and hydroxyl groups, as well as their positions, on the molecules are important characteristics that determine the effectiveness of the aromatic compounds in their inhibition of Aβ aggregation (Lakey-Beitia et al. 2014; Begum et al. 2008).

One class of aromatic compounds, flavonoids, has garnered increasing interest in the prevention and treatment of AD. Flavonoids are plant secondary metabolites and are a subclass of the naturally-occurring polyphenols. Flavonoids have long been known for their multi-faceted pharmacology, including anti-inflammatory, anti-oxidant, anti-tumorigenic, anti-cancer, anti-microbial, and anti-vascular properties (Ringman et al. 2005; Lakey-Beitia et al. (2014); Kang et al. 2005). The diverse range of biological properties appears to stem from the ability of this class of compounds to bind to a multitude of cellular targets, and thereby regulate multiple cellular pathways (Ringman et al. 2005; Ramassamy 2006). Moreover, the beneficial effects of flavonoids in neurodegenerative diseases have been documented (Ganguli et al. 2000; Gao et al. 2012), although understanding the molecular mechanisms by which flavonoids exert their biological effects remains an active research area. We summarize below the known beneficial roles of flavonoids in AD, with an emphasis on dimeric flavonoids, the biflavonoids. As flavonoids are naturally occurring and an integral part of a diet rich in fruits and vegetables, they are safe, with little or no adverse side effects, and abundant. Additionally, flavonoids are able to target multiple risk factors of AD, including inflammation, oxidation, Aβ aggregation, and Aβ toxicity, these compounds are particularly promising therapeutics for both the prevention as well as treatment of AD.

3.4 Beneficial Roles of Naturally Occurring Polyphenolic Compounds in AD

Polyphenols comprise naturally occurring phenyl ring-containing small molecules found in fruits and vegetables and have been shown to offer beneficial effects in AD and other neurodegenerative disorders (Ringman et al. 2005; Lakey-Beitia et al. 2014; Kang et al. 2005). Although their mode of action and targets in these diseases are not yet clear, the phenyl ring structure is believed to be central to polyphenol's beneficial effects (Yang et al. 2005; Gazit 2002). Polyphenols perform a myriad of biological functions to improve the health of the organisms and ac-

tivate a multitude of pharmacological pathways (Gauci et al. 2011; Wang et al. 2001; Ramassamy 2006; Hamaguchi et al. 2009). More than 8,000 naturally occurring polyphenols, ranging from simple small molecules to highly polymerized compounds, have been identified and characterized. The compounds are efficiently absorbed in the intestine and do not have any adverse side effects (Ringman et al. 2005).

A number of pathways have been identified by which polyphenols may exert their protective role in AD. The compounds are capable of efficiently chelating heavy metal ions (Leopoldini et al. 2006; Yang et al. 2005), thereby reducing a primary source of free radicals in neurons that contribute to oxidative stress (Cuajungco et al. 2000). In addition to being chelating agents, polyphenols are also antioxidants as the hydroxyl groups are capable of consuming free radicals and terminating redox reactions that cause cellular damages. The polyphenols myricetin and quercetin have been found to alleviate inflammation, which is also one of the features of AD (Lee and Choi 2010; Boots et al. 2011). The polyphenols curcumin, epigallocatechin gallate, and resveratrol have been found to effectively reduce Aβ toxicity *in vitro* and *in vivo* by multiple mechanisms (Yang et al. 2005; Feng et al. 2009). Strikingly, the beneficial effects of these compounds have been found to correlate with the delay in the onset of AD and other aging related diseases in the elderly. In addition to their protective role in AD, polyphenols such as quercetin have also been reported to be anti-microbial, exerting toxicity towards many different strains of pathogenic bacteria (Su et al. 2014). Polyphenols from black tea, e.g. catechins and theaflavin, also possess anti-fungal activities (Betts et al. 2013). Furthermore, the polyphenols apigenin, kaemferol, and catechin have been found to exhibit anti-viral and anti-cancer properties (Zhang et al. 2004; Pang et al. 2014).

Aside from the beneficial pharmacological effects described above, polyphenols also interfere with the π-π stacking between aromatic residue side chains of Aβ peptides, suggesting that these compounds may affect Aβ aggregation as well (Re et al. 2010; Gazit 2002). The presence of phenyl groups and internal double bonds, the ability to form hydrogen bonds, and molecular planarity are some of the other features of the polyphenols that may be involved in modulating Aβ aggregation. A study based on the polyphenol curcumin's structure-function relationship have indicated that the two terminal phenyl groups, the substituted aromatic end groups that participate in hydrogen bonding, and an optimal rigid linker length of 6–19 Å, are critical structural features that inhibit Aβ aggregation (Yang et al. 2005). It is also suggested that a flat and planar molecule with two aromatic end groups and substituted aromatic groups are suitable ligands for Aβ peptides and may be neuroprotective (Yang et al. 2005; Reinke and Gestwicki 2007).

Flavonoids are a class of polyphenolic compounds with a C6-C3-C6 backbone (Fig. 3.1a) that adopt a flat and planar structure (Thapa et al. 2011; Re et al. 2010). Flavonoids consist of two aromatic rings (α and β) linked with a pyran ring (γ) and are the largest group of polyphenols available from dietary fruits and vegetables (Lakey-Beitia et al. 2014; Re et al. 2010). The chemical structures of flavonoids are quite diverse. Based on the oxidation state of the pyran ring, flavonoids are classified into flavones, flavonols, flavanols, isoflavonoids, flavanones, and anthocyanins (Lakey-Beitia et al. 2014; Re et al. 2010). Hydroxylation of flavonoids occurs mainly at the C5, C7 and C4′ positions (Fig. 3.1a). Flavones such as apigenin and luteolin are rich in parsley and celery. Flavonols possess a keto-hydroxypyrene group, which is hydroxylated at the C3, C5 and C7 positions (Fig. 3.1a). Myricetin and quercetin are examples of flavonols that are found in onions and broccoli. Flavanols have a hydroxylated pyran ring mostly at the C3 position and are present in green tea and red wine. Soy product flavonoids such as genistein and daidzein are categorized as isoflavonoids, which have a substituted keto group on a pyran ring structure. The flavanone group consisting of hesperetin and naringenin has substituted keto-pyran groups on ring α at C3 and C5 positions and are abundant in citrus and tomatoes. Anthocyanin found in berries offers the colors in the plant and fruits. Non-flavonoid types of polyphenols include: (i) phenolic acids (e.g. caffeic acid and gallic acid

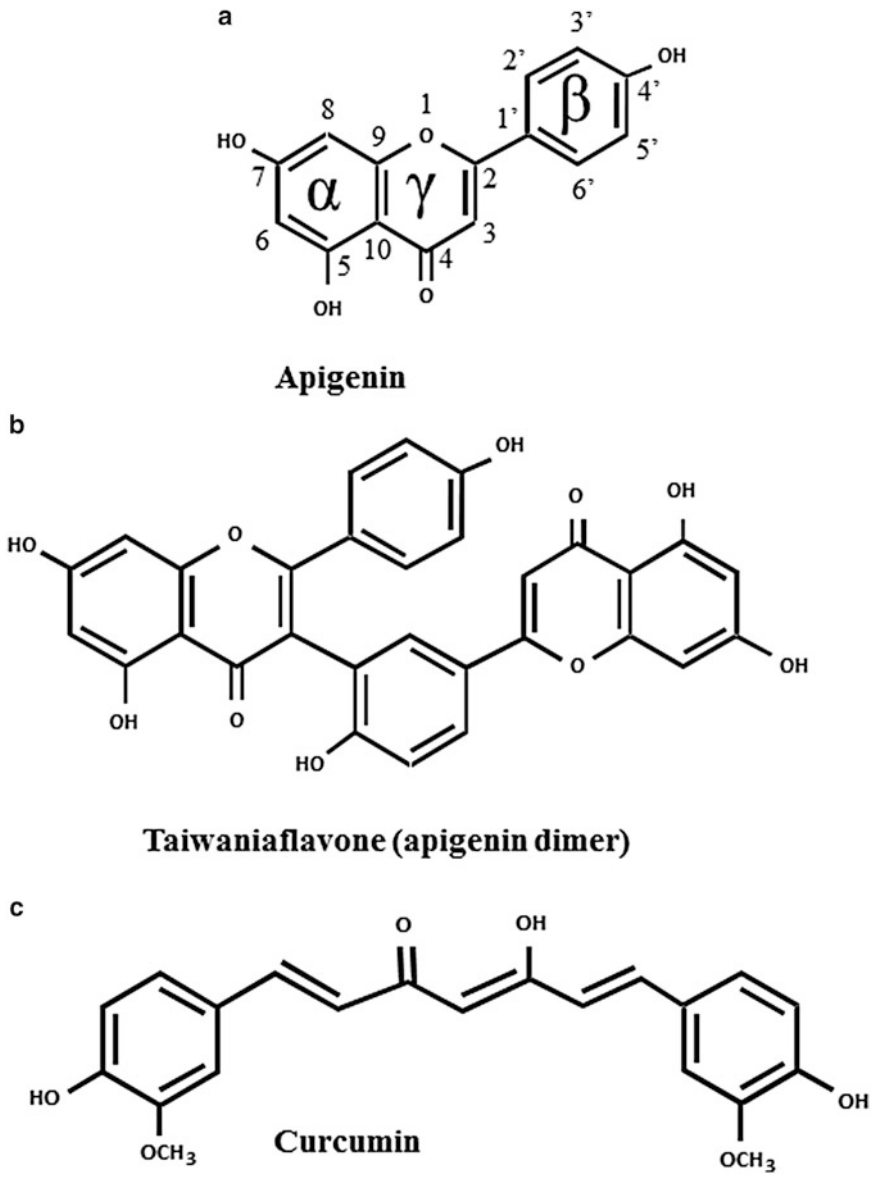


Fig. 3.1 Schematics of monoflavonoid apigenin (**a**), biflavonoid taiwaniaflavone (apigenin dimer, apigenin-3 and 3‴-apigenin) (**b**), and curcumin (**c**). Phenol rings are denoted as α-, β-, and γ (Reprinted with permission from Thapa et al. 2011)

etc.), (ii) lignans (e.g. secoisolariciresinol), (iii) stilbenes (e.g. resveratrol), and (iv) curcuminoids (e.g. curcumin, Fig. 3.1c) (Lakey-Beitia et al. 2014).

As a subclass of polyphenols, flavonoids exhibit the same wide range of biological functions, including anti-oxidative, anti-inflammatory, anti-tumorigenic, and anti-amyloidogenic activities (Ramassamy 2006; Lakey-Beitia et al. 2014). Recent studies have shown that larger flavonoid compounds that contain multiple chemical moieties, for example, dimers of single flavonoid structures or biflavonoids, show enhanced biological activities compared to their monomeric flavonoid counterparts (Zhang et al. 2004; Wong et al.

2007; Dhawan et al. 2002). For example, the flavonoid taiwaniaflavone (Fig. 3.1b) is a dimer of the flavonoid apigenin (Fig. 3.1a). Apigenin is a known anti-cancer agent with moderate activity in multidrug resistance breast cancer (Zhang et al. 2004). Dimers of apigenin linked with ethylene glycol linker showed enhanced anti-cancer effect compared to apigenin in multidrug resistance breast cancer cells as well as in leukemia cell lines (Zhang et al. 2004). It was suggested that dimeric molecules could bind to two distinct sites on a single molecule or a defined site on two separate molecules (Hadden and Blagg 2008). With respect to Aβ peptide aggregation and toxicity, a dimeric polyphenol, curcumin (Fig. 3.1c), has been shown to potently inhibit Aβ aggregation and toxicity and improve cognitive function (Yang et al. 2005). Studies on curcumin also indicated that a flat and planar shaped polyphenol molecule with two aromatic end groups and substituted aromatic groups may be necessary for inhibiting Aβ aggregation and toxicity. This conclusion was further supported by the observation that ferulic acid, which is structurally similar to one-half of curcumin, does not inhibit Aβ aggregation. However, a dimeric form of ferulic acid effectively inhibits Aβ aggregation (Byeon et al. 2007). The polyphenol that contains both ferulic acid and styryl benzene has been shown to be more effective than either of the two individual compounds (Lee et al. 2005). It has been further suggested that the dimers of Aβ binding monoflavonoids, separated by an appropriate size linker, may serve as a general structure for inhibitors of Aβ aggregation and toxicity (Thapa et al. 2011). Such biflavonoid compounds may be promising therapeutic candidates for AD.

3.5 Beneficial Roles of Biflavonoids in Alzheimer's Disease

As discussed earlier, biflavonoids are flavonoid-flavonoid dimers connected by a C-C or a C-O-C linkage (Fig. 3.1b). Naturally occurring biflavonoids contain different combinations of monoflavonoids, such as flavone-flavone, flavone-flavonol, or flavanone-flavone (Lakey-Beitia et al. 2014; Kim et al. 2008). Biflavonoids are abundant in the *Ginkgo biloba*, *Selaginella*, and *Garcinia* plant species. This subclass of polyphenols exhibits multiple biological and pharmacological properties stemming from the different types of chemical substitutions, including hydroxyl and methoxyl groups. The anti-microbial action of the biflavonoid amentoflavone and its synergistic effect with antibiotics have been documented (Hwang et al. 2013). Amentoflavone stimulates mitochondrial dysfunction and induces apoptotic cell death in yeast (Hwang et al. 2012). The biflavonoid ginkgetin is cytotoxic to human ovarian adenocarcinoma and is also effective against the flu virus (Kim et al. 2008; Miki et al. 2007). We review below the potent anti-oxidant, anti-inflammatory, anti-acetylcholinesterase, β-secretase inhibiting, and anti-amyloidogenic properties of biflavonoids that have been reported to contribute to their beneficial effects in AD.

3.5.1 Biflavonoids as Anti-oxidants

Free radicals are among the normal cellular metabolic byproducts but their accumulation over time is undesirable as they contribute to oxidative stress. Free radicals attack proteins, lipids, and nucleic acids and disrupt their structures and functions. One of the best-known properties of flavonoids is their anti-oxidative capability. Virtually all flavonoids are able to scavenge reactive oxygen species and terminate the detrimental redox reactions. The biflavonoid morelloflavone is known as a potent lipid-peroxidation inhibitor (Gil et al. 1997). It is believed that biflavonoids could more efficiently neutralize free radicals compared to monoflavonoids due to the presence of the additional hydroxyl groups. Further, flavonoids are capable of removing metal ions bound to proteins and lipids; for example, the flavonoid quercetin has been found to chelate heavy metals (Leopoldini et al. 2006). The use of naturally occurring flavonoids, particularly biflavonoids,

for chelating metal ions and scavenging free radicals appears to be an attractive approach to prevent AD.

3.5.2 Biflavonoids as Anti-inflammatory Agents

Brain inflammation is another common feature of AD. The stimulation of pro-inflammatory cytokines, such as interleukin-1 and interferon-γ, results in the production of large amounts of reactive nitric oxides by inducing nitric oxide synthase (iNOS), which could lead to neuronal death (Nuzzo et al. 2014; Pokharel et al. 2006). Inflammation mediators such as prostaglandins that are involved in chronic inflammation are metabolic products of cyclooxygenase (COX) and the pharmacological inhibition of COX and iNOS results in the relief of pain and inflammation. Biflavonoids such as amentoflavone, taiwaniaflavone, bilobetin, and ginkgetin have been found to down regulate iNOS and COX-2 expression, and are known to regulate pro-inflammatory molecules at the transcription level (Pokharel et al. 2006; Kim et al. 1998, 2008).

3.5.3 Biflavonoids as Secretase Inhibitors

Flavonoids have also been viewed as an attractive class of molecules to limit Aβ production by altering the activities of secretases. The monoflavonoid myricetin has been shown to reduce Aβ production in cell-free systems as well as in neuronal cells (Shimmyo et al. 2008a). They appear to reduce Aβ production by upregulating α-secretase activity and/or down regulating β-secretase activity (Shimmyo et al. 2008a, b). For reasons that are still unclear, other monoflavonoids that are structurally similar to myricetin such as apigenin and morin were found to be less effective at reducing β-secretase activity. On the other hand, a study on β-secretase inhibition has revealed that amentoflavone-type biflavonoids (molar mass 600 Da) potently inhibited β-secretase activity (Sasaki et al. 2010). It is believed that biflavoniods, being roughly twice the size of monoflavonoids, better fit the large sized β-secretase catalytic cleft to inhibit β-secretase activity compared to smaller sized monoflavonoids (Ghosh et al. 2012). More recently, the polyphenol tannic acid, a naturally occurring non-flavonoid polymer (molar mass 1,700 Da) was found to inhibit β-secretase activity, prevent cognitive impairment, and reduce AD-like pathology in transgenic mice (Mori et al. 2012). However, due to its large size, tannic acid is impermeable to the BBB (molecular weight limit for BBB is believed to be 600 Da). From these studies, it appears that intermediate-sized biflavonoid molecules may be effective and BBB-permeable candidates for modulating secretase activity.

Although the flavonoids that have exhibited beneficial effects in various aspects of AD are multifunctional and have been shown to reduce oxidative-stress, inflammation, and Aβ production, the roles they play in the disease process and the interplay between these effects in AD remain to be explored. For instance, some studies have shown that oxidative stress and inflammatory processes are secondary events in AD. As the vast majority of AD cases are sporadic rather than familial, it is reasonable to conclude that Aβ aggregation rather than Aβ production is the primary pathogenic event in AD (Pimplikar 2009; Walsh and Selkoe 2007; Chromy et al. 2003). Modulating secretase activities that reduce Aβ production, therefore, is expected to have only a limited effect on Aβ aggregation. Removal of the triggers of Aβ aggregation, e.g., oxidative stress, or direct disruption of the aggregation process itself, are also needed in order to achieve complete inhibition of Aβ aggregation, and thereby AD pathogenesis.

3.5.4 Biflavonoids as Aβ Aggregation Inhibitors

Studies that have investigated small molecule inhibitors of Aβ aggregation suggest that the inhibitor should strongly interact with Aβ (Nie et al. 2011). Additionally, the inhibitor should

have sufficient length and flexibility, which would enable them to capture the entire Aβ peptide and accommodate conformational changes in the target (Nie et al. 2011). Thus, an effective Aβ-aggregation inhibitor should contain moieties that strongly interact with Aβ, be of appropriate size, and exhibit suitable flexibility. In this regard, naturally occurring biflavonoids containing two or more flavonoid units may be a better option to target Aβ aggregation as compared to simpler polyphenols and flavonoids. It is possible that small polyphenols, including monoflavonoids, may not produce sufficient steric hindrance as necessary to interact with and inhibit Aβ aggregation as compared to biflavonoids. Thus, flavonoids with multiple phenol groups that are sufficiently long and flexible, such as biflavonoids, hold greater potential for inhibiting Aβ aggregation and toxicity, and thereby prevent AD (Reinke and Gestwicki 2007). However, there are substantial challenges associated with utilizing biflavonoids as aggregation inhibitors since their effects vary greatly even with minor chemical substitutions (Ono et al. 2003, 2006; Shimmyo et al. 2008a). A clear understanding of their aggregation inhibitory effects at the molecular level has yet to emerge.

Aβ peptides spontaneously aggregate *in vitro* at concentrations above its critical micelle concentration. Aβ fibril formation can be easily detected with the Thioflavin-T fluorescence assay, as the dye binds to β-sheet rich Aβ fibrils and upon binding, the fluorescence of the dye at 480 nm is greatly enhanced upon excitation at 445 nm. When incubated alone in solution, the formation of Aβ fibrils follows a sigmoidal curve, which represents a nucleation-dependent Aβ polymerization mechanism. A lag phase during which fibril nuclei are formed followed by a rapid fiber extension phase are typical features of this aggregation process (Naiki et al. 1998). The final plateaued level of fluorescence in this model is indicative of the complete conversion of monomeric Aβ into fibrils. A study that compared the effects of the monoflavonoid apigenin and biflavonoid taiwaniaflavone on Aβ fibril formation showed that the biflavonoid reduced the final level of Thioflavin-T fluorescence to a greater extent compared to the monoflavonoid (Thapa et al. 2011). The study also showed that neither the monoflavonoids nor the biflavonoids significantly affected the nucleation step. Seeding experiments, where predominantly monomeric Aβ samples were seeded with preformed fibrils to eliminate the nucleation phase, were also carried out to explore the effects of the flavonoids on Aβ fibril extension kinetics. Initial rates of fibril extension in the presence of different concentrations of a monoflavonoid and a biflavonoid are shown in Fig. 3.2. As shown, both flavonoids decreased the rate of fibril extension with increasing flavonoid concentration. A saturation effect was apparent for both flavonoids as the decrease in rate plateaued at high flavonoid concentrations, indicating binding saturation of the flavonoids to Aβ species. Strikingly, the biflavonoid reduced the rate of fibril extension to a much greater extent compared to the monoflavonoid and its saturation effect is also less pronounced than that of the monoflavonoid (Fig. 3.2). Thus, the biflavonoid was found to be more effective at reducing Aβ fibril extension compared to the monoflavonoid (Thapa et al. 2011).

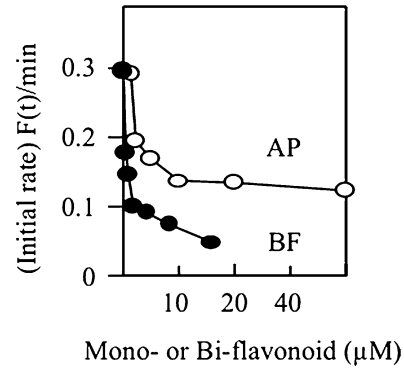


Fig. 3.2 The initial rate of Aβ42 fiber extension in the presence or absence of biflavonoid taiwaniaflavone (•, BF) or monoflavonoid apigenin (○, AP) obtained by extrapolating Thioflavin-T florescence data (Reprinted with permission from Thapa et al. 2011)

3.5.5 Biflavonoids as Inhibitors of β-Sheet Formation

The structural transition of the intrinsically unstructured Aβ peptides into β-sheet-enriched fibrils is a characteristic feature of the Aβ aggregation process. Disruption of β-sheet formation can potentially inhibit Aβ aggregation and fibril formation. The far UV-circular dichroism (CD) spectrum, which gives information regarding protein secondary structural content, of monomeric Aβ peptides in solution exhibited negative ellipticity at 190 nm, indicating a random coil structure (Fig. 3.3). Upon aggregation, the CD spectrum of Aβ changed and showed negative ellipiticity at 217 nm, indicating the presence of β-sheet structures (Fig. 3.3) (Thapa et al. 2008). Aβ aggregates formed in the presence of biflavonoids contained predominantly random coil structures, whereas aggregates formed in the presence of monoflavonoids show a similar level of β-sheet structure compared to Aβ aggregates formed in the absence of flavonoids (Fig. 3.3). These findings suggest that while smaller flavonoid molecules such as monoflavonoids had little effect on the β-sheet formation of Aβ, larger flavonoid molecules such as biflavonoids can perturb β-sheet formation by Aβ peptides. The β-sheet structure blocking/disrupting capability of the biflavonoids also provides an explanation to its observed inhibitory effect on Aβ fibril extension as described in the previous section (Thapa et al. 2011).

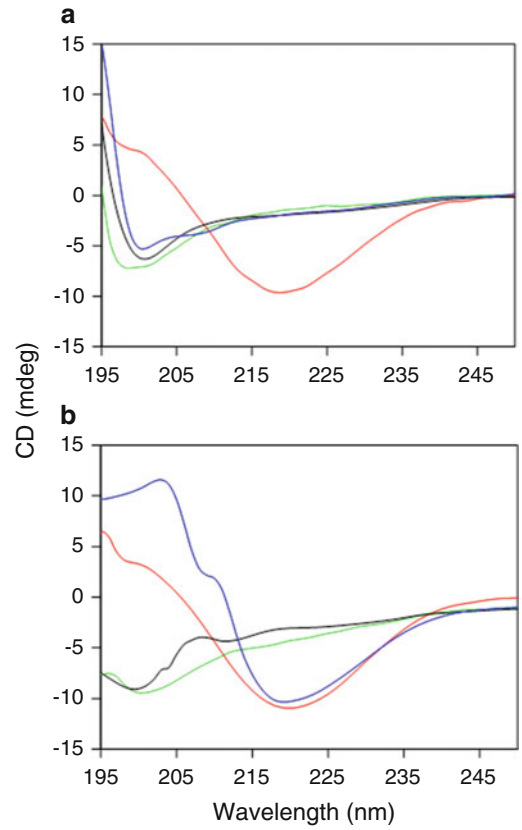


Fig. 3.3 Circular dichroism (CD) spectra of 20 μM Aβ42 incubated under different conditions. (**a**): Aβ incubated alone for 0 h (*green*) or 12 h (*red*) and Aβ incubated with 20 μM biflavonoid taiwaniaflavone for 0 h (*black*) or 12 h (*blue*). (**b**): Aβ incubated alone for 0 h (*green*) or 12 h (*red*) and Aβ incubated with 20 μM monoflavonoid apigenin for 0 h (*black*) or 12 h (*blue*) (Reprinted with permission from Thapa et al. 2011)

3.5.6 Biflavonoids as Specific Inhibitors of Aβ-Induced Toxicity

The broad-spectrum biological activities of polyphenols and flavonoids, including anti-oxidant, anti-microbial, anti-cancer, anti-inflammatory, and metal-chelating activities, are now well known. How some of these biological activities, such as anti-oxidant, metal chelating, and anti-inflammatory effects, contribute towards the beneficial roles polyphenols and flavonoids play in AD and other aging related illnesses are also becoming more clear (Shin et al. 2006; Kim et al. 2008; You et al. 2013). In addition to these known effects, many reports have documented that polyphenols can directly mediate Aβ aggregation as well as ameliorate Aβ-induced toxicity. However, these effects vary and a consensus regarding their molecular mechanisms has yet to emerge.

A number of Aβ aggregation inhibitors that have been identified (see Sect. 3.3.8) are polyphenols and some are flavonoids. However, despite their anti-amyloidgenic activity, many of the compounds do not exhibit a neuroprotective

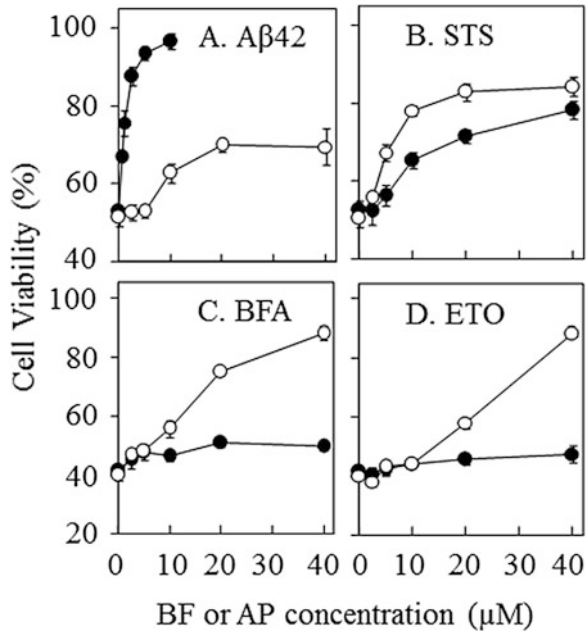


Fig. 3.4 Percentage cell viability of human neuroblastoma SH-SY5Y cells treated with 0.5 μM predominantly monomeric Aβ42 (*A*: Aβ42), staurosporine (*B*: STS), brefeldin A (*C*: BFA), and etoposide (*D*: ETO) in the presence or absence of varying concentrations of biflavonoid taiwaniaflavone (•, BF) or monoflavonoid apigenin (∘, AP). Cells were treated for 12 h in DMEM/F12 containing media without phenol red and fetal bovine serum. Cell viability was determined by MTT assay. Data are presented as the mean ± standard deviation from 3 to 4 independent experimental measurements (Reprinted with permission from Thapa et al. 2011)

effect (Thapa et al. 2011; Ramassamy 2006; Ono et al. 2003, 2006). The flavonoid quercetin inhibits Aβ fibril formation to a similar extent compared to myricetin, but it is less effective in ameliorating Aβ toxicity compared to myricetin, which possesses an additional -OH group (Ono et al. 2003). Some polyphenols, including flavonoids, have been reported to inhibit Aβ aggregation but not Aβ-induced toxicity, while others have been found to be neuroprotective without significantly reducing Aβ aggregation (Ono et al. 2003; Wang et al. 2001; Feng et al. 2009; Sharoar et al. 2012). While it is difficult to distinguish and establish the specific roles these multifunctional polyphenols, flavonoids, and biflavonoid molecules play in a complex disease such as AD, some studies have shown that biflavonoids are more effective at inhibiting Aβ aggregation and reducing Aβ-induced toxicity and may be a better class of naturally-occurring compounds to target AD. We review below recent publications on the effects and proposed neuroprotective mechanisms of biflavonoids on Aβ-induced toxicity.

A study comparing the effects of monoflavonoids and biflavonoids on Aβ-induced toxicity in cultured neuronal cells has revealed some insights into the neuroprotective properties of these two types of flavonoids (Thapa et al. 2011). In agreement with other published reports, flavonoids were shown to rescue cells exposed to monomeric Aβ42 peptides (Thapa et al. 2011). However, the effects of the biflavonoids and monoflavonoids are distinctly different. The study showed that while the presence of 10 μM monoflavonoid apigenin increased the viability of cultured SH-SY5Y neuroblastoma cell by 50 % when exposed to 0.5 μM of monomeric Aβ42 for 12 h, only approximately 1 μM of the biflavonoid was required to achieve the same level of neuroprotection (Fig. 3.4) (Thapa et al. 2011).

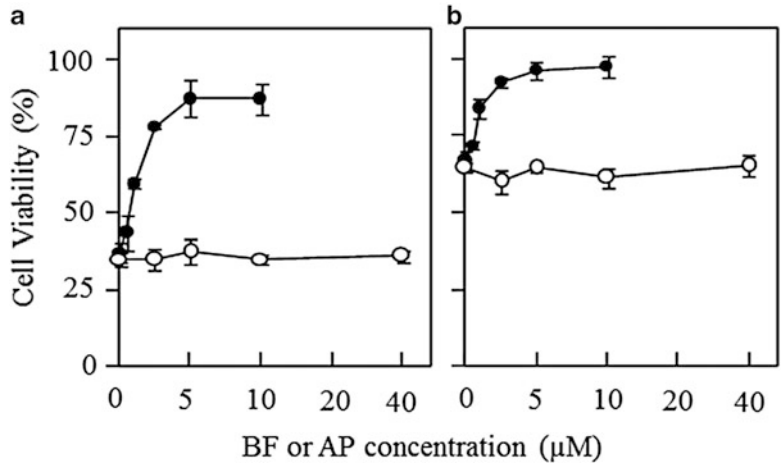


Fig. 3.5 Percentage cell viability of human neuroblastoma SH-SY5Y cells treated with 0.5 μM preformed Aβ42 oligomers (**a**) or fibrils (**b**) in the presence or absence of biflavonoid taiwaniaflavone (•, BF) or monoflavonoid apigenin (∘, AP). Cells were treated for 12 h in DMEM/F12 containing media without phenol red and fetal bovine serum. Cell viability was determined by MTT assay. Data are presented as the mean ± standard deviation from 3 to 4 independent experimental measurements (Reprinted with permission from Thapa et al. 2011)

To test the specificity of the rescuing effects of the flavonoids towards Aβ-induced toxicity, the effects of the two types of flavonoids on the viability of cultured SH-SY5Y cells exposed to a number of known toxic compounds including staurosporine (an inhibitor of protein kinase), brefildin A (a protein transport inhibitor to Golgi complex), and etoposide (an inhibitor of DNA topoisomerase) were also studied (Karki et al. 2007; Shin et al. 2006). The monoflavonoid apigenin was found to be moderately effective in increasing the viability of cells exposed to Aβ and was found to be equally effective at rescuing cells exposed to the other toxic compounds. Previously, it was shown that apigenin abrogates the activation of the executioner molecule caspase-3 in the apoptotic cell death cascade (Wang et al. 2001). Such a cytoprotective mechanism explains the moderate and non-specific rescuing effect that has been observed for the monoflavonoid. In contrast to the nonspecific rescuing effect of monoflavonoids, biflavonoids rescued cells to a greater extent towards toxicity induced by monomeric Aβ42 compared to toxicity induced by the other compounds (Fig. 3.4). These data suggest that biflavonoids more specifically and more effectively reduce toxicity induced by Aβ peptide compared to monoflavonoids (Fig. 3.4). The enhanced rescuing effects can arise from biflavonoid's ability to either more potently inhibit Aβ peptide aggregation during the incubation period with the cells (Figs. 3.2 and 3.3) or that biflavonoids are more effective at directly blocking monomeric Aβ42 toxicity.

In a separate study, the neuroprotective effects of mono- and bi-flavonoids against oligomeric and fibrillar Aβ42 were examined. In contrast to monomeric Aβ42, the soluble Aβ42 oligomers are known to be significantly more toxic and are believed to be the primary neurotoxic species in the Aβ peptide aggregation cascade (Deshpande et al. 2006; Walsh and Selkoe 2007). As shown in Fig. 3.5, preformed 0.5 μM Aβ42 oligomers induced nearly 16 % more cell death compared to monomeric Aβ42. The biflavonoid taiwaniaflavone significantly increased the viability of cells exposed to preformed Aβ oligomers and fibrils whereas the monoflavonoid apigenin did not offer neuroprotection against Aβ oligomers and fibrils (Fig. 3.5). These findings suggest that the biflavonoids are uniquely effective at inhibiting the toxicity of soluble Aβ oliogmers (Fig. 3.5) and are therefore promising small molecule therapeutics for the prevention of Aβ toxicity and neurodegeneration in AD.

3.5.7 Biflavonoids Accumulate Nontoxic Aβ Oligomers

Since soluble Aβ oligomers are believed to be the primary toxic species of Aβ causing neurodegeneration, small molecules, including flavonoids, that selectively bind to Aβ oligomers over monomeric of fibrillar Aβ are considered as potential therapeutics for targeting and neutralizing Aβ toxicity in AD (Walsh and Selkoe 2007; Klein 2002; Yang et al. 2005). Several other small molecules that inhibit Aβ aggregation have also been shown as inhibitors of Aβ oligomerization and/or Aβ fibril formation (Necula et al. 2007). A study on biflavonoids has revealed that these compounds efficiently capture small Aβ oligomers and reduce subsequent Aβ aggregation (Thapa et al. 2011). Immunoblotting analysis of gluteraldehyde cross-linked Aβ42 aggregates formed by incubating Aβ alone showed the presence of high molecular weight Aβ species along with the disappearance of monomeric Aβ. Analysis of soluble Aβ aggregates formed in the presence of the biflavonoid taiwaniaflavone showed that significantly more low-molecular-weight (LMW) Aβ42 aggregates (including monomeric, dimeric, and trimeric Aβ42) were present, along with small amounts of high-molecular-weight (HMW) soluble oligomers spanning a broad size range. Moreover, the levels of LMW Aβ aggregates and HMW Aβ oligomers formed in the presence of the biflavonoid increased in a dose-dependent manner (Fig. 3.6). In contrast, Aβ aggregates formed in the presence of the monoflavonoid apigenin did not contain LMW Aβ aggregates; the oligomers that were present were larger than 30 kDa (Fig. 3.6). Similar results were also obtained from studies that used other biflavonoids, including 2′,8″-biapigenin, amentoflavone, sumaflavone (Thapa et al. 2011). These results suggest that biflavonoids uniquely and selectively bind to soluble LMW Aβ oligomers. As a result, these Aβ oligomers are thermodynamically favored, leading to their increased levels. Critically, these small and soluble Aβ oligomers bound to biflavonoids have been shown to be nontoxic and unstructured. The Aβ aggregates formed in the presence of biflavonoids were thus structurally and functionally different than on-pathway toxic Aβ aggregates (Thapa et al. 2011).

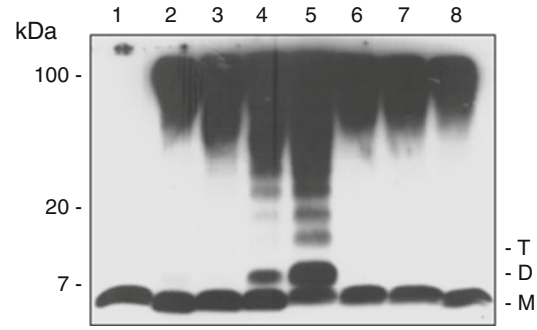


Fig. 3.6 Western blot analysis of 0.5 μM Aβ incubated alone or in the presence of biflavonoid taiwaniaflavone or monoflavonoid apigenin. Lane-1: Aβ incubated alone; lane-2: Aβ incubated for 3 h; lanes 3–5: Aβ incubated in the presence of taiwaniaflavone (2.5, 5 and 10 μM) for 3 h; lanes 6–8: Aβ incubated in the presence of apigenin (2.5, 5 and 10 μM) for 3 h. Aβ incubated in PBS at 37 °C was cross-linked with 0.01 % (v/v) gluteraldehyde before separating on 16 % acrylamide gel. The samples were then transferred onto PVDF membrane and probed with Aβ specific 6E10 monoclonal antibody at 1:10,000 dilution. Monomer, dimer, and trimer bands are indicated by M, D and T, respectively (Reprinted with permission from Thapa et al. 2011)

3.5.8 Mechanisms of Biflavonoid-Mediated Neuroprotection Against Aβ-Induced Toxicity

Elucidating the molecular mechanisms by which polyphenols affect Aβ aggregation and toxicity remains an active research area. Several recently published reports gave useful insights to the mechanisms by which polyphenols and flavonoids antagonize Aβ-induced toxicity. In one study, Necula and co-workers investigated the effects of a large number of small molecules on the oligomerization and fibrillization of Aβ42. Results from the study showed that the small molecules could be categorized into three groups based on their inhibitory effects on Aβ42 aggregation. One group was found to be effective at inhibiting the oligomerization

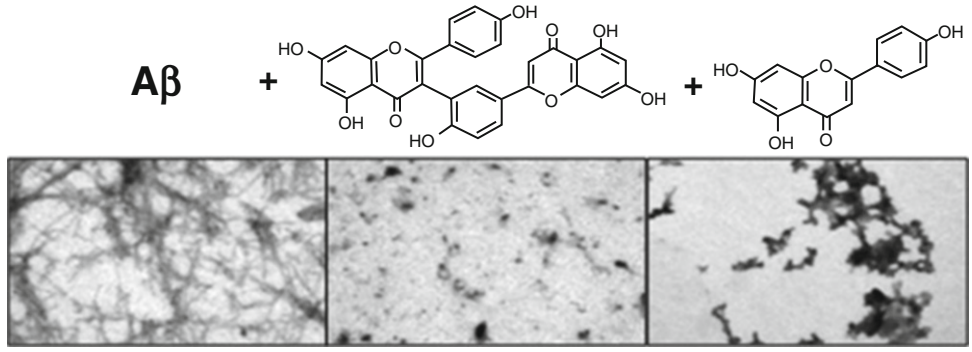


Fig. 3.7 Schematics of mono- and bi-flavonoids (*top*) and TEM images (*bottom*) of Aβ incubated alone (*left image*) or in the presence of biflavonoid taiwaniaflavone (*middle image*) or monoflavonoid apigenin (*right image*) (Reprinted with permission from Thapa et al. 2011)

of Aβ while another group was found to be effective at inhibiting the fibrillization of Aβ. The third group of small molecules were effective at inhibiting both fibrillization and oligomerization of Aβ (Necula et al. 2007). In a separate study, Ladiwala and co-workers investigated the effects of polyphenolic compounds on Aβ42 aggregation and classified the aromatic molecules according to their abilities to (i) remodel soluble oligomers into non-toxic off-pathway aggregates, (ii) convert soluble Aβ oligomers into fibrils, and (iii) disaggregate soluble oligomers or fibrils into non-toxic, LMW species (Ladiwala et al. 2011).

In a study that specifically examined the effects of biflavonoids on Aβ42 aggregation kinetics, Thapa and co-workers showed that the compounds primarily affected the fibril extension phase of Aβ aggregation. Biochemical analysis further revealed that biflavonoids captured soluble Aβ aggregates more efficiently than monoflavonoids (Fig. 3.6) (Thapa et al. 2011). Moreover, transmission electron microscopy (TEM) imaging of Aβ aggregates showed that Aβ incubated alone contained predominantly fibrillar aggregates whereas the Aβ sample incubated with biflavonoids contained small globular oligomeric aggregates (Fig. 3.7). In contrast, beaded protofibril-like Aβ aggregates were found in the Aβ sample incubated with monoflavonoids. Because the biflavonoids, but not the monoflavonoids, were found to be neuroprotective against preformed Aβ oligomers and fibrils in this study, it was concluded that the oligomers accumulated by the biflavonoids are non-toxic (Fig. 3.6). This study shows that while the biflavonoids are effective at reducing Aβ fibril extension, they are also able to direct the Aβ aggregation pathway towards a non-toxic pathway, as has been proposed for the neuroprotective mechanism of tea polyphenols (Ehrnhoefer et al. 2008).

3.6 Conclusion

Sporadic AD represents over 95 % of all AD cases (Hamley 2012; Thies and Bleiler 2013). The dominant prevalence of sporadic over familial AD indicates that reduction of Aβ production by manipulating secretases that cleave APP may only have a limited effect in preventing and treating AD (Nie et al. 2011). It also highlights the fact that Aβ aggregation is critical in AD development; hence, inhibiting or modifying Aβ aggregation is a highly promising AD therapeutic strategy. Because the soluble oligomers and protofibrils formed during Aβ fibril formation are believed to be the primary toxic species and their toxicity correlates well with the progression of AD, the inhibition of Aβ aggregation, especially at an early stage that inhibits the formation of toxic oligomers, appears to be a promising preventive strategy for AD (Ehrnhoefer et al. 2008; Ladiwala et al. 2010; Tomic et al. 2009).

Unfortunately, a number of clinical trials that have been conducted in the past few years, including heavy metal chelators, oxidative

stress reducers, and anti-inflammatory inhibitors, have yielded largely unsatisfactory outcomes (Blennow et al. 2006; Nie et al. 2011). Perhaps an important lesson that emerges from these trials is that compounds that have multiple modes of action against AD development, especially those that inhibit Aβ aggregation, may be promising drug candidates for the prevention and treatment of AD.

Polyphenols are a class of naturally occurring compounds with well-documented anti-oxidant and anti-inflammatory properties. There is increasing interest in utilizing these compounds as AD therapeutics as they have also been shown to be capable of disrupting hydrophobic and π-π interactions of Aβ peptides and prevent Aβ aggregation and toxicity (Armstrong et al. 2011; Gazit 2002). Studies on the structure-function relationship of polyphenols revealed that a minimum of two phenyl rings connected by a suitably long and flexible linker are essential features that contribute to aggregation inhibition (Reinke and Gestwicki 2007). In this regard, the flat and planar biflavonoid compounds that are endowed with multiple phenyl ring moieties connected by appropriately sized and flexible linkers may target multiple and specific regions on the Aβ peptide in a concerted manner. Biflavonoids have indeed been shown to exert a stronger aggregation inhibition effect compared to the smaller-sized monoflavonoids, possibly due to their higher affinity to Aβ and the greater steric hindrance for fibril growth when bound to Aβ peptides. These properties are believed to contribute to the enhanced anti-amyloidgenic activity of biflavonoids, resulting in reduced Aβ toxicity. More importantly, biflavonoids have been shown to reduce Aβ oligomers toxicity more specifically while capturing soluble Aβ oligomers and reducing Aβ aggregation.

These unique and potent biochemical and biological activities of biflavonoids suggest that they may be better candidate small molecules in preventing AD as compared to polyphenols, including monomeric flavonoid, in AD interventions. Further studies in an *in vivo* setting using transgenic mouse models of AD are therefore urgently warranted.

References

Armstrong AH, Chen J, McKoy AF, Hecht MH (2011) Mutations that replace aromatic side chains promote aggregation of the Alzheimer's Abeta peptide. Biochemistry 50(19):4058–4067. doi:10.1021/bi200268w

Begum AN, Jones MR, Lim GP, Morihara T, Kim P, Heath DD, Rock CL, Pruitt MA, Yang F, Hudspeth B, Hu S, Faull KF, Teter B, Cole GM, Frautschy SA (2008) Curcumin structure-function, bioavailability, and efficacy in models of neuroinflammation and Alzheimer's disease. J Pharmacol Exp Ther 326(1):196–208

Betts JW, Wareham DW, Haswell SJ, Kelly SM (2013) Antifungal synergy of theaflavin and epicatechin combinations against Candida albicans. J Microbiol Biotechnol 23(9):1322–1326

Blennow K, de Leon MJ, Zetterberg H (2006) Alzheimer's disease. Lancet 368(9533):387–403

Boots AW, Drent M, de Boer VC, Bast A, Haenen GR (2011) Quercetin reduces markers of oxidative stress and inflammation in sarcoidosis. Clin Nutr 30(4):506–512. doi:10.1016/j.clnu.2011.01.010

Brogi S, Butini S, Maramai S, Colombo R, Verga L, Lanni C, De Lorenzi E, Lamponi S, Andreassi M, Bartolini M, Andrisano V, Novellino E, Campiani G, Brindisi M, Gemma S (2014) Disease-modifying anti-alzheimer's drugs: inhibitors of human cholinesterases interfering with beta-amyloid aggregation. CNS Neurosci Ther 20(7):624–632. doi:10.1111/cns.12290

Butterfield DA, Drake J, Pocernich C, Castegna A (2001) Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid beta-peptide. Trends Mol Med 7(12):548–554

Byeon SR, Jin YJ, Lim SJ, Lee JH, Yoo KH, Shin KJ, Oh SJ, Kim DJ (2007) Ferulic acid and benzothiazole dimer derivatives with high binding affinity to beta-amyloid fibrils. Bioorg Med Chem Lett 17(14):4022–4025

Chauhan V, Chauhan A (2006) Oxidative stress in Alzheimer's disease. Pathophysiology 13(3):195–208

Chen YR, Glabe CG (2006) Distinct early folding and aggregation properties of Alzheimer amyloid-beta peptides Abeta40 and Abeta42: stable trimer or tetramer formation by Abeta42. J Biol Chem 281(34):24414–24422

Chromy BA, Nowak RJ, Lambert MP, Viola KL, Chang L, Velasco PT, Jones BW, Fernandez SJ, Lacor PN, Horowitz P, Finch CE, Krafft GA, Klein WL (2003) Self-assembly of Abeta(1–42) into globular neurotoxins. Biochemistry 42(44):12749–12760

Cuajungco MP, Faget KY, Huang X, Tanzi RE, Bush AI (2000) Metal chelation as a potential therapy for Alzheimer's disease. Ann N Y Acad Sci 920:292–304

Dasilva KA, Shaw JE, McLaurin J (2010) Amyloid-beta fibrillogenesis: structural insight and therapeutic intervention. Exp Neurol 223(2):311–321. doi:10.1016/j.expneurol.2009.08.032

Delrieu J, Ousset PJ, Caillaud C, Vellas B (2012) 'Clinical trials in Alzheimer's disease': immunotherapy ap-

proaches. J Neurochem 120(Suppl 1):186–193. doi:10.1111/j.1471-4159.2011.07458.x

Deshpande A, Mina E, Glabe C, Busciglio J (2006) Different conformations of amyloid beta induce neurotoxicity by distinct mechanisms in human cortical neurons. J Neurosci 26(22):6011–6018

Dhawan A, Anderson D, de Pascual-Teresa S, Santos-Buelga C, Clifford MN, Ioannides C (2002) Evaluation of the antigenotoxic potential of monomeric and dimeric flavanols, and black tea polyphenols against heterocyclic amine-induced DNA damage in human lymphocytes using the Comet assay. Mutat Res 515(1–2):39–56

Doody RS, Raman R, Farlow M, Iwatsubo T, Vellas B, Joffe S, Kieburtz K, He F, Sun X, Thomas RG, Aisen PS, Siemers E, Sethuraman G, Mohs R (2013) A phase 3 trial of semagacestat for treatment of Alzheimer's disease. N Engl J Med 369(4):341–350. doi:10.1056/NEJMoa1210951

Ehrnhoefer DE, Bieschke J, Boeddrich A, Herbst M, Masino L, Lurz R, Engemann S, Pastore A, Wanker EE (2008) EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers. Nat Struct Mol Biol 15(6):558–566

Estrada LD, Soto C (2007) Disrupting beta-amyloid aggregation for Alzheimer disease treatment. Curr Top Med Chem 7(1):115–126

Feng Y, Wang XP, Yang SG, Wang YJ, Zhang X, Du XT, Sun XX, Zhao M, Huang L, Liu RT (2009) Resveratrol inhibits beta-amyloid oligomeric cytotoxicity but does not prevent oligomer formation. Neurotoxicology 30(6):986–995

Findeis MA (2007) The role of amyloid beta peptide 42 in Alzheimer's disease. Pharmacol Ther 116(2):266–286

Funke SA, Willbold D (2012) Peptides for therapy and diagnosis of Alzheimer's disease. Curr Pharm Des 18(6):755–767

Ganguli M, Chandra V, Kamboh MI, Johnston JM, Dodge HH, Thelma BK, Juyal RC, Pandav R, Belle SH, DeKosky ST (2000) Apolipoprotein E polymorphism and Alzheimer disease: the Indo-US cross-national dementia study. Arch Neurol 57(6):824–830

Gao X, Cassidy A, Schwarzschild MA, Rimm EB, Ascherio A (2012) Habitual intake of dietary flavonoids and risk of Parkinson disease. Neurology 78(15):1138–1145. doi:10.1212/WNL.0b013e31824f7fc4

Gasparini L, Ongini E, Wenk G (2004) Non-steroidal anti-inflammatory drugs (NSAIDs) in Alzheimer's disease: old and new mechanisms of action. J Neurochem 91(3):521–536

Gauci AJ, Caruana M, Giese A, Scerri C, Vassallo N (2011) Identification of polyphenolic compounds and black tea extract as potent inhibitors of lipid membrane destabilization by Abeta(4)(2) aggregates. J Alzheimers Dis 27(4):767–779. doi:10.3233/JAD-2011-111061

Gazit E (2002) A possible role for pi-stacking in the self-assembly of amyloid fibrils. FASEB J 16(1):77–83

Ghosh AK, Brindisi M, Tang J (2012) Developing beta-secretase inhibitors for treatment of Alzheimer's disease. J Neurochem 120(Suppl 1):71–83. doi:10.1111/j.1471-4159.2011.07476.x

Gil B, Sanz MJ, Terencio MC, Gunasegaran R, Paya M, Alcaraz MJ (1997) Morelloflavone, a novel biflavonoid inhibitor of human secretory phospholipase A2 with anti-inflammatory activity. Biochem Pharmacol 53(5):733–740

Gu F, Zhu M, Shi J, Hu Y, Zhao Z (2008) Enhanced oxidative stress is an early event during development of Alzheimer-like pathologies in presenilin conditional knock-out mice. Neurosci Lett 440(1):44–48

Haass C, Hung AY, Schlossmacher MG, Oltersdorf T, Teplow DB, Selkoe DJ (1993) Normal cellular processing of the beta-amyloid precursor protein results in the secretion of the amyloid beta peptide and related molecules. Ann N Y Acad Sci 695:109–116

Hadden MK, Blagg BS (2008) Dimeric approaches to anti-cancer chemotherapeutics. Anti Cancer Agents Med Chem 8(7):807–816

Hamaguchi T, Ono K, Murase A, Yamada M (2009) Phenolic compounds prevent Alzheimer's pathology through different effects on the amyloid-beta aggregation pathway. Am J Pathol 175(6):2557–2565

Hamley IW (2012) The amyloid beta peptide: a chemist's perspective. Role in Alzheimer's and fibrillization. Chem Rev 112(10):5147–5192. doi:10.1021/cr3000994

Hane F, Tran G, Attwood SJ, Leonenko Z (2013) Cu(2+) affects amyloid-beta (1–42) aggregation by increasing peptide-peptide binding forces. PLoS One 8(3):e59005. doi:10.1371/journal.pone.0059005

Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297(5580):353–356

Hwang IS, Lee J, Jin HG, Woo ER, Lee DG (2012) Amentoflavone stimulates mitochondrial dysfunction and induces apoptotic cell death in Candida albicans. Mycopathologia 173(4):207–218. doi:10.1007/s11046-011-9503-x

Hwang JH, Choi H, Woo ER, Lee DG (2013) Antibacterial effect of amentoflavone and its synergistic effect with antibiotics. J Microbiol Biotechnol 23(7):953–958

Jay GW, Demattos RB, Weinstein EJ, Philbert MA, Pardo ID, Brown TP (2011) Animal models for neural diseases. Toxicol Pathol 39(1):167–169. doi:10.1177/0192623310389478

Johnston GA, Hanrahan JR, Chebib M, Duke RK, Mewett KN (2006) Modulation of ionotropic GABA receptors by natural products of plant origin. Adv Pharmacol 54:285–316

Kang SS, Lee JY, Choi YK, Song SS, Kim JS, Jeon SJ, Han YN, Son KH, Han BH (2005) Neuroprotective effects of naturally occurring biflavonoids. Bioorg Med Chem Lett 15(15):3588–3591

Karki P, Seong C, Kim JE, Hur K, Shin SY, Lee JS, Cho B, Park IS (2007) Intracellular K(+) inhibits apoptosis by suppressing the Apaf-1 apoptosome formation and

subsequent downstream pathways but not cytochrome c release. Cell Death Differ 14(12):2068–2075
Kim HK, Son KH, Chang HW, Kang SS, Kim HP (1998) Amentoflavone, a plant biflavone: a new potential anti-inflammatory agent. Arch Pharm Res 21(4):406–410
Kim HP, Park H, Son KH, Chang HW, Kang SS (2008) Biochemical pharmacology of biflavonoids: implications for anti-inflammatory action. Arch Pharm Res 31(3):265–273
Klein WL (2002) Abeta toxicity in Alzheimer's disease: globular oligomers (ADDLs) as new vaccine and drug targets. Neurochem Int 41(5):345–352
Ladiwala AR, Lin JC, Bale SS, Marcelino-Cruz AM, Bhattacharya M, Dordick JS, Tessier PM (2010) Resveratrol selectively remodels soluble oligomers and fibrils of amyloid Abeta into off-pathway conformers. J Biol Chem 285(31):24228–24237. doi:10.1074/jbc.M110.133108
Ladiwala AR, Dordick JS, Tessier PM (2011) Aromatic small molecules remodel toxic soluble oligomers of amyloid beta through three independent pathways. J Biol Chem 286(5):3209–3218. doi:10.1074/jbc.M110.173856
LaFerla FM, Green KN (2012) Animal models of Alzheimer disease. Cold Spring Harb Perspect Med 2(11). doi:10.1101/cshperspect.a006320
Lahiri DK, Maloney B, Long JM, Greig NH (2014) Lessons from a BACE1 inhibitor trial: off-site but not off base. Alzheimers Dement 10(5 Suppl):S411–S419. doi:10.1016/j.jalz.2013.11.004
Lakey-Beitia J, Berrocal R, Rao KS, Durant AA (2014) Polyphenols as therapeutic molecules in Alzheimer's disease through modulating Amyloid pathways. Mol Neurobiol. doi:10.1007/s12035-014-8722-9
Lee YS, Choi EM (2010) Myricetin inhibits IL-1beta-induced inflammatory mediators in SW982 human synovial sarcoma cells. Int Immunopharmacol 10(7):812–814. doi:10.1016/j.intimp.2010.04.010
Lee KH, Shin BH, Shin KJ, Kim DJ, Yu J (2005) A hybrid molecule that prohibits amyloid fibrils and alleviates neuronal toxicity induced by beta-amyloid (1–42). Biochem Biophys Res Commun 328(4):816–823
Leopoldini M, Russo N, Chiodo S, Toscano M (2006) Iron chelation by the powerful antioxidant flavonoid quercetin. J Agric Food Chem 54(17):6343–6351
Lyketsos CG, Breitner JC, Green RC, Martin BK, Meinert C, Piantadosi S, Sabbagh M (2007) Naproxen and celecoxib do not prevent AD in early results from a randomized controlled trial. Neurology 68(21):1800–1808
Mason JM, Kokkoni N, Stott K, Doig AJ (2003) Design strategies for anti-amyloid agents. Curr Opin Struct Biol 13(4):526–532
Miki K, Nagai T, Suzuki K, Tsujimura R, Koyama K, Kinoshita K, Furuhata K, Yamada H, Takahashi K (2007) Anti-influenza virus activity of biflavonoids. Bioorg Med Chem Lett 17(3):772–775
Mori T, Rezai-Zadeh K, Koyama N, Arendash GW, Yamaguchi H, Kakuda N, Horikoshi-Sakuraba Y, Tan J, Town T (2012) Tannic acid is a natural beta-secretase inhibitor that prevents cognitive impairment and mitigates Alzheimer-like pathology in transgenic mice. J Biol Chem 287(9):6912–6927. doi:10.1074/jbc.M111.294025
Naiki H, Hasegawa K, Yamaguchi I, Nakamura H, Gejyo F, Nakakuki K (1998) Apolipoprotein E and antioxidants have different mechanisms of inhibiting Alzheimer's beta-amyloid fibril formation in vitro. Biochemistry 37(51):17882–17889
Necula M, Kayed R, Milton S, Glabe CG (2007) Small molecule inhibitors of aggregation indicate that amyloid beta oligomerization and fibrillization pathways are independent and distinct. J Biol Chem 282(14):10311–10324
Nie Q, Du XG, Geng MY (2011) Small molecule inhibitors of amyloid beta peptide aggregation as a potential therapeutic strategy for Alzheimer's disease. Acta Pharmacol Sin 32(5):545–551. doi:10.1038/aps.2011.14
Nuzzo D, Picone P, Caruana L, Vasto S, Barera A, Caruso C, Di Carlo M (2014) Inflammatory mediators as biomarkers in brain disorders. Inflammation 37(3):639–648. doi:10.1007/s10753-013-9780-2
Ono K, Yoshiike Y, Takashima A, Hasegawa K, Naiki H, Yamada M (2003) Potent anti-amyloidogenic and fibril-destabilizing effects of polyphenols in vitro: implications for the prevention and therapeutics of Alzheimer's disease. J Neurochem 87(1):172–181
Ono K, Hamaguchi T, Naiki H, Yamada M (2006) Anti-amyloidogenic effects of antioxidants: implications for the prevention and therapeutics of Alzheimer's disease. Biochim Biophys Acta 1762(6):575–586
Pallitto MM, Ghanta J, Heinzelman P, Kiessling LL, Murphy RM (1999) Recognition sequence design for peptidyl modulators of beta-amyloid aggregation and toxicity. Biochemistry 38(12):3570–3578
Pang JY, Zhao KJ, Wang JB, Ma ZJ, Xiao XH (2014) Green tea polyphenol, epigallocatechin-3-gallate, possesses the antiviral activity necessary to fight against the hepatitis B virus replication in vitro. J Zhejiang Univ Sci B 15(6):533–539. doi:10.1631/jzus.B1300307
Permanne B, Adessi C, Saborio GP, Fraga S, Frossard MJ, Van Dorpe J, Dewachter I, Banks WA, Van Leuven F, Soto C (2002) Reduction of amyloid load and cerebral damage in a transgenic mouse model of Alzheimer's disease by treatment with a beta-sheet breaker peptide. FASEB J 16(8):860–862
Pike CJ, Walencewicz AJ, Glabe CG, Cotman CW (1991) Aggregation-related toxicity of synthetic beta-amyloid protein in hippocampal cultures. Eur J Pharmacol 207(4):367–368
Pimplikar SW (2009) Reassessing the amyloid cascade hypothesis of Alzheimer's disease. Int J Biochem Cell Biol 41(6):1261–1268
Poduslo JF, Curran GL, Kumar A, Frangione B, Soto C (1999) Beta-sheet breaker peptide inhibitor of Alzheimer's amyloidogenesis with increased blood-brain barrier permeability and resistance to proteolytic degradation in plasma. J Neurobiol 39(3):371–382

Pokharel YR, Yang JW, Kim JY, Oh HW, Jeong HG, Woo ER, Kang KW (2006) Potent inhibition of the inductions of inducible nitric oxide synthase and cyclooxygenase-2 by taiwaniaflavone. Nitric Oxide 15(3):217–225

Porat Y, Abramowitz A, Gazit E (2006) Inhibition of amyloid fibril formation by polyphenols: structural similarity and aromatic interactions as a common inhibition mechanism. Chem Biol Drug Des 67(1):27–37

Ramassamy C (2006) Emerging role of polyphenolic compounds in the treatment of neurodegenerative diseases: a review of their intracellular targets. Eur J Pharmacol 545(1):51–64

Rasool S, Martinez-Coria H, Wu JW, LaFerla F, Glabe CG (2013) Systemic vaccination with anti-oligomeric monoclonal antibodies improves cognitive function by reducing Abeta deposition and tau pathology in 3xTg-AD mice. J Neurochem 126(4):473–482. doi:10.1111/jnc.12305

Re F, Airoldi C, Zona C, Masserini M, La Ferla B, Quattrocchi N, Nicotra F (2010) Beta amyloid aggregation inhibitors: small molecules as candidate drugs for therapy of Alzheimer's disease. Curr Med Chem 17(27):2990–3006

Reinke AA, Gestwicki JE (2007) Structure-activity relationships of amyloid beta-aggregation inhibitors based on curcumin: influence of linker length and flexibility. Chem Biol Drug Des 70(3):206–215

Ringman JM, Frautschy SA, Cole GM, Masterman DL, Cummings JL (2005) A potential role of the curry spice curcumin in Alzheimer's disease. Curr Alzheimer Res 2(2):131–136

Rosini M, Simoni E, Minarini A, Melchiorre C (2014) Multi-target design strategies in the context of Alzheimer's disease: acetylcholinesterase inhibition and NMDA receptor antagonism as the driving forces. Neurochem Res 39(10):1914–1923. doi:10.1007/s11064-014-1250-1

Roychaudhuri R, Yang M, Hoshi MM, Teplow DB (2009) Amyloid beta-protein assembly and Alzheimer disease. J Biol Chem 284(8):4749–4753

Sasaki H, Miki K, Kinoshita K, Koyama K, Juliawaty LD, Achmad SA, Hakim EH, Kaneda M, Takahashi K (2010) beta-Secretase (BACE-1) inhibitory effect of biflavonoids. Bioorg Med Chem Lett 20(15):4558–4560. doi:10.1016/j.bmcl.2010.06.021

Schimmer AD, Jitkova Y, Gronda M, Wang Z, Brandwein J, Chen C, Gupta V, Schuh A, Yee K, Chen J, Ackloo S, Booth T, Keays S, Minden MD (2012) A phase I study of the metal ionophore clioquinol in patients with advanced hematologic malignancies. Clin Lymphoma Myeloma Leuk 12(5):330–336. doi:10.1016/j.clml.2012.05.005

Selkoe DJ (1991) The molecular pathology of Alzheimer's disease. Neuron 6(4):487–498

Sharoar MG, Thapa A, Shahnawaz M, Ramasamy VS, Woo ER, Shin SY, Park IS (2012) Keampferol-3-O-rhamnoside abrogates amyloid beta toxicity by modulating monomers and remodeling oligomers and fibrils to non-toxic aggregates. J Biomed Sci 19:104. doi:10.1186/1423-0127-19-104

Shimmyo Y, Kihara T, Akaike A, Niidome T, Sugimoto H (2008a) Flavonols and flavones as BACE-1 inhibitors: structure-activity relationship in cell-free, cell-based and in silico studies reveal novel pharmacophore features. Biochim Biophys Acta 1780(5):819–825

Shimmyo Y, Kihara T, Akaike A, Niidome T, Sugimoto H (2008b) Multifunction of myricetin on A beta: neuroprotection via a conformational change of A beta and reduction of A beta via the interference of secretases. J Neurosci Res 86(2):368–377

Shin DH, Bae YC, Kim-Han JS, Lee JH, Choi IY, Son KH, Kang SS, Kim WK, Han BH (2006) Polyphenol amentoflavone affords neuroprotection against neonatal hypoxic-ischemic brain damage via multiple mechanisms. J Neurochem 96(2):561–572

Soto C, Saborio GP, Permanne B (2000) Inhibiting the conversion of soluble amyloid-beta peptide into abnormally folded amyloidogenic intermediates: relevance for Alzheimer's disease therapy. Acta Neurol Scand Suppl 176:90–95

Su Y, Ma L, Wen Y, Wang H, Zhang S (2014) Studies of the in vitro antibacterial activities of several polyphenols against clinical isolates of methicillin-resistant Staphylococcus aureus. Molecules 19(8):12630–12639. doi:10.3390/molecules190812630

Suh YH (1997) An etiological role of amyloidogenic carboxyl-terminal fragments of the beta-amyloid precursor protein in Alzheimer's disease. J Neurochem 68(5):1781–1791

Thapa A, Shahnawaz M, Karki P, Raj Dahal G, Sharoar MG, Yub Shin S, Sup Lee J, Cho B, Park IS (2008) Purification of inclusion body-forming peptides and proteins in soluble form by fusion to Escherichia coli thermostable proteins. Biotechniques 44(6):787–796

Thapa A, Woo ER, Chi EY, Sharoar MG, Jin HG, Shin SY, Park IS (2011) Biflavonoids are superior to monoflavonoids in inhibiting amyloid-beta toxicity and fibrillogenesis via accumulation of nontoxic oligomer-like structures. Biochemistry 50(13):2445–2455. doi:10.1021/bi101731d

Thies W, Bleiler L (2013) 2013 Alzheimer's disease facts and figures. Alzheimers Dement 9(2):208–245. doi:10.1016/j.jalz.2013.02.003

Tomic JL, Pensalfini A, Head E, Glabe CG (2009) Soluble fibrillar oligomer levels are elevated in Alzheimer's disease brain and correlate with cognitive dysfunction. Neurobiol Dis 35(3):352–358

Uversky VN (2010) Mysterious oligomerization of the amyloidogenic proteins. FEBS J 277(14):2940–2953. doi:10.1111/j.1742-4658.2010.07721.x

Walsh DM, Selkoe DJ (2007) A beta oligomers – a decade of discovery. J Neurochem 101(5):1172–1184

Walsh DM, Hartley DM, Kusumoto Y, Fezoui Y, Condron MM, Lomakin A, Benedek GB, Selkoe DJ, Teplow DB (1999) Amyloid beta-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. J Biol Chem 274(36):25945–25952

Wang CN, Chi CW, Lin YL, Chen CF, Shiao YJ (2001) The neuroprotective effects of phytoestrogens on amyloid beta protein-induced toxicity are mediated by abrogating the activation of caspase cascade in rat cortical neurons. J Biol Chem 276(7):5287–5295

Wong IL, Chan KF, Burkett BA, Zhao Y, Chai Y, Sun H, Chan TH, Chow LM (2007) Flavonoid dimers as bivalent modulators for pentamidine and sodium stiboglucanate resistance in leishmania. Antimicrob Agents Chemother 51(3):930–940

Wu Z, Guo Z, Gearing M, Chen G (2014) Tonic inhibition in dentate gyrus impairs long-term potentiation and memory in an Alzhiemer's disease model. Nat Commun 5:4159. doi:10.1038/ncomms5159

Yang F, Lim GP, Begum AN, Ubeda OJ, Simmons MR, Ambegaokar SS, Chen PP, Kayed R, Glabe CG, Frautschy SA, Cole GM (2005) Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid in vivo. J Biol Chem 280(7):5892–5901

Yang SG, Wang WY, Ling TJ, Feng Y, Du XT, Zhang X, Sun XX, Zhao M, Xue D, Yang Y, Liu RT (2010) Alpha-tocopherol quinone inhibits beta-amyloid aggregation and cytotoxicity, disaggregates preformed fibrils and decreases the production of reactive oxygen species, NO and inflammatory cytokines. Neurochem Int 57(8):914–922. doi:10.1016/j.neuint.2010.09.011

You OH, Kim SH, Kim B, Sohn EJ, Lee HJ, Shim BS, Yun M, Kwon BM, Kim SH (2013) Ginkgetin induces apoptosis via activation of caspase and inhibition of survival genes in PC-3 prostate cancer cells. Bioorg Med Chem Lett 23(9):2692–2695. doi:10.1016/j.bmcl.2013.02.080

Yu JT, Tan L, Hardy J (2014) Apolipoprotein E in Alzheimer's disease: an update. Annu Rev Neurosci 37:79–100. doi:10.1146/annurev-neuro-071013-014300

Zhang S, Yang X, Morris ME (2004) Combined effects of multiple flavonoids on breast cancer resistance protein (ABCG2)-mediated transport. Pharm Res 21(7):1263–1273

Zhou WW, Lu S, Su YJ, Xue D, Yu XL, Wang SW, Zhang H, Xu PX, Xie XX, Liu RT (2014) Decreasing oxidative stress and neuroinflammation with a multifunctional peptide rescues memory deficits in mice with Alzheimer's disease. Free Radic Biol Med 74:50–63. doi:10.1016/j.freeradbiomed.2014.06.013

Natural Phenolic Compounds as Therapeutic and Preventive Agents for Cerebral Amyloidosis

4

Masahito Yamada, Kenjiro Ono, Tsuyoshi Hamaguchi, and Moeko Noguchi-Shinohara

Abstract

Epidemiological studies have suggested that diets rich in phenolic compounds may have preventive effects on the development of dementia or Alzheimer's disease (AD). We investigated the effects of natural phenolic compounds, such as myricetin (Myr), rosmarinic acid (RA), ferulic acid (FA), curcumin (Cur) and nordihydroguaiaretic acid (NDGA) on the aggregation of amyloid β-protein (Aβ), using *in vitro* and *in vivo* models of cerebral Aβ amyloidosis. The *in vitro* studies revealed that these phenolic compounds efficiently inhibit oligomerization as well as fibril formation of Aβ through differential binding, whilst reducing Aβ oligomer-induced synaptic and neuronal toxicity. Furthermore, a transgenic mouse model fed orally with such phenolic compounds showed significant reduction of soluble Aβ oligomers as well as of insoluble Aβ deposition in the brain. These data, together with an updated review of the literature, indicate that natural phenolic compounds have anti-amyloidogenic effects on Aβ in addition to well-known anti-oxidative and anti-inflammatory effects, hence suggesting their potential as therapeutic and/or preventive agents for cerebral Aβ amyloidosis, including AD and cerebral amyloid angiopathy (CAA). Well-designed clinical trials or preventive interventions with natural phenolic compounds are necessary to establish their efficacy as disease-modifying agents.

Keywords

Alzheimer's disease • Amyloid β-protein • Amyloidosis • Polyphenols • Therapy

Abbreviations

Aβ	amyloid β-protein
AD	Alzheimer's disease
ADAS-Cog	Alzheimer's Disease Assessment Scale – Cognitive Subscale

M. Yamada, M.D., Ph.D. (✉) • K. Ono • T. Hamaguchi
M. Noguchi-Shinohara
Department of Neurology and Neurobiology of Aging, Kanazawa University Graduate School of Medical Sciences, 13-1, Takara-machi, Kanazawa 920-8640, Japan
e-mail: m-yamada@med.kanazawa-u.ac.jp

N. Vassallo (ed.), *Natural Compounds as Therapeutic Agents for Amyloidogenic Diseases*, Advances in Experimental Medicine and Biology 863, DOI 10.1007/978-3-319-18365-7_4

αS	α-synuclein
ApoE	apolipoprotein E
APP	amyloid-β precursor protein
CAA	cerebral amyloid angiopathy
CD	circular dichroism
CSF	cerebrospinal fluid
Cur	curcumin
DLB	dementia with Lewy bodies
EGCG	(−)-epigallocatechin-3-galate
FA	ferulic acid
fAβ	Aβ fibrils
GSPE	grape seed polyphenolic extract
LTD	long-term depression
LTP	long-term potentiation
MCI	mild cognitive impairment
MMSE	Mini-Mental State Examination
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
Myr	myricetin
NDGA	nordihydroguaiaretic acid
NMR	nuclear magnetic resonance
PD	Parkinson's disease
PHF	paired helical filament
PICUP	photo-induced cross-linking of unmodified proteins
RA	rosmarinic acid

4.1 Epidemiological Studies Suggesting Preventive Effects of Phenol Compound-Rich Diets on Dementia or Alzheimer's Disease

Epidemiological studies have reported that diets rich in phenolic compounds or polyphenols may be associated with a reduced risk of dementia or Alzheimer's disease (AD). These include vegetables, fruits, spice, and derived products such as wine and non-alcoholic beverages.

The Mediterranean diet, characterized by a high intake of vegetables, fruits, cereals, olive oil, fish, in combination with a low intake of meat and poultry, was reported to be associated with a reduction in risk of dementia, mild cognitive impairment (MCI), and AD in prospective longitudinal studies (Scarmeas et al. 2006, 2009; Féart et al. 2009). In a randomized trial with nutritional intervention comparing two Mediterranean diets supplemented with either extra-virgin olive oil or nuts versus a low-fat control diet for 6.5 years, cognitive performance examined by Mini-Mental State Examination (MMSE) and Clock Drawing Test was significantly better in the group of Mediterranean diets than in a low-fat control group, after adjusting multiple confounding factors (Martínez-Lapiscina et al. 2013). Recent systematic reviews with meta-analysis indicate that a higher adherence to Mediterranean diet is associated with a reduced risk of MCI, dementia, and AD; nevertheless, further prospective cohort studies with longer follow-up and randomized controlled trials are necessary to unequivocally establish the effects of this type of diet on cognitive decline and AD (Psaltopoulou et al. 2013; Singh et al. 2014).

Traditional Indian diets and medicines contain spices such as yellow curry spice turmeric, curcumin. Frequency of AD in India is about one-quarter of that in the US (aged 70–79 years, 0.7 % vs 3.1 %; aged 80 years or older, 4.0 % vs 15.7 %), suggesting influence of ethnic differences in environmental, including dietary, apart from genetic factors (Ganguli et al. 2000). In a population-based cohort of non-demented elderly Asian subjects, more curry consumption was associated with better cognitive performance suggesting possible preventive effects of curry spice curcumin on cognitive decline, although further prospective cohort studies with long follow-up are required (Ng et al. 2006).

Several prospective cohort studies also reported that moderate intake of wine was associated with a reduced risk of dementia, AD, or cognitive decline (Orgogozo et al. 1997; Truelsen et al. 2002; Luchsinger et al. 2004; Solfrizzi et al. 2007; Mehlig et al. 2008; Arntzen et al. 2010). As this protective effect was not seen for alcoholic beverages other than wine (Truelsen et al. 2002; Luchsinger et al. 2004; Mehlig et al. 2008; Arntzen et al. 2010), it is suggested that

the association for wine may be attributable to components of wine other than ethanol itself. In a population-based prospective study, consumption of fruit and vegetable juices, containing a high concentration of polyphenols, decreased a risk of AD (Dai et al. 2006).

Coffee, black tea, and green tea are enriched in polyphenols, and may be protective against onset of dementia including AD. Several longitudinal studies (Lindsay et al. 2002; van Gelder et al. 2007; Ritchie et al. 2007; Eskelinen et al. 2009) have investigated the relationship between coffee consumption and dementia, AD, or cognitive decline, but findings from these studies are inconsistent. Longitudinal studies of black tea consumption have not found any association with reduced risks for dementia, AD, or cognitive decline (Laurin et al. 2004; Dai et al. 2006). One cross-sectional study has shown that higher green tea consumption is associated with lower prevalence of cognitive impairment (Kuriyama et al. 2006). To determine whether the consumption of green tea, coffee, or black tea influences the incidence of dementia and MCI in older people, we recently conducted a population-based prospective study with Japanese residents aged >60 years from Nakajima, Japan (the Nakajima Project) (Noguchi-Shinohara et al. 2014). Participants received an evaluation of cognitive function and blood tests. The consumption of green tea, coffee, and black tea was also evaluated at baseline. Of 723 participants with normal cognitive function at a baseline survey (2007–2008), 490 completed the follow up survey in 2011–2013. The incidence of dementia during the follow-up period (mean ± SD: 4.9 ± 0.9 years) was 5.3 %, and that of MCI was 13.1 %. To analyze the independent effects of green tea, coffee, and black tea consumption on the risk of developing dementia or MCI, multivariate logistic regression analysis was performed with adjustment for sex, age, history of hypertension, diabetes mellitus, and hyperlipidemia, formal education, apolipoprotein E (*ApoE*) phenotype status (*ApoE* E4+ or E4-), smoking status, alcohol consumption, green tea, coffee, and/or black tea consumption, physical activities and/or hobbies. The multiple-adjusted odds ratio for the incidence of overall cognitive decline (dementia or MCI) was 0.32 (95 % CI: 0.16–0.64) among individuals who consumed green tea every day and 0.47 (95 % CI: 0.25–0.86) among those who consumed green tea 1–6 days per week compared with individuals who did not consume green tea at all (Fig. 4.1). No association was found between coffee or black tea consumption and the incidence of dementia or MCI. Our results indicate that green tea consumption is significantly associated with reduced risk of cognitive decline, even after adjustment for possible confounding factors. This was the first prospective longitudinal study that examined the association between green tea consumption and incidence of dementia or cognitive decline.

Figure 4.2 shows components of green tea, black tea, and coffee with possible effects on cognitive decline. The major tea-related polyphenols present in green tea are catechins, especially (−)-epigallocatechin-3-galate (EGCG), whereas black tea mainly contains theaflavins (Peterson et al. 2005). In addition, green tea contains greater amounts of myricetin (Myr) compared with black tea (Peterson et al. 2005). Other tea-related polyphenols such as quercetin, kaempferol, apigenin, and luteolin, are also present in both green and black tea, but the amounts of these polyphenols are not significantly different between tea types (Peterson et al. 2005). The caffeine content is 40–57 mg/100 mL in coffee (Barone and Roberts 1996), 25.5 mg/100 mL in black tea, and only 15.3 mg/100 mL in green tea (Khokhar and Magnusdottir 2002). High intake of ascorbic acid was reported to be associated with lower risk of AD (Engelhart et al. 2002). The content of ascorbic acid is 6 mg/100 mL in green tea, which is the most common source of ascorbic acid in Japan (Ogawa et al. 2002); on the other hand, coffee and black tea do not contain ascorbic acid. As the serum levels of ascorbic acid were associated with the frequency of coffee consumption, but not green tea consumption in our study (Noguchi-Shinohara et al. 2014), it is unlikely that the effects of green tea on cognitive function could be explained as those of ascorbic acid. Taken together, phenolic compounds enriched in green

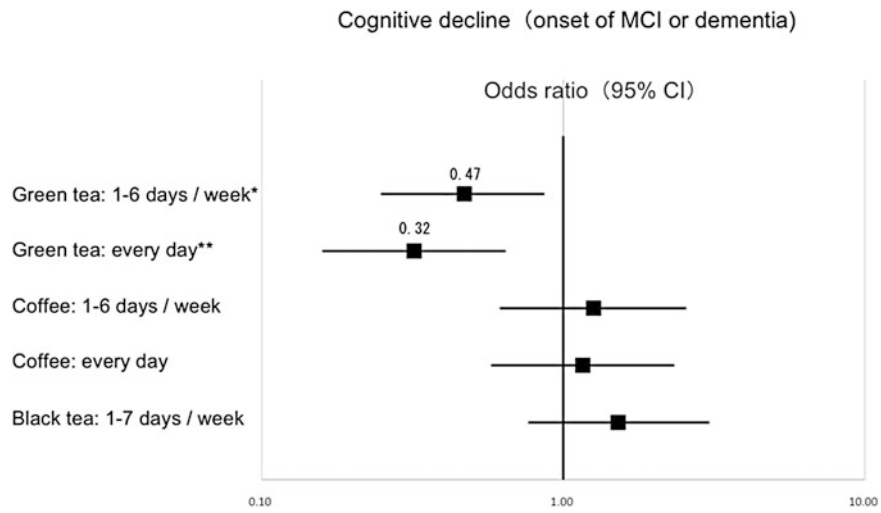


Fig. 4.1 Association between green tea, coffee, or black tea consumption and the incidence of cognitive decline [mild cognitive impairment (MCI) or dementia] in cognitively normal subjects (age > 60 years) ($n = 490$) during the follow-up period (mean ± SD: 4.9 ± 0.9 years). The multiple-adjusted odds ratio[#] for the incidence of cognitive decline (MCI or dementia) is shown compared with individuals who did not consume green tea, coffee, or black tea at all. Details were reported in the reference (Noguchi-Shinohara et al. 2014) [#]Multivariate logistic regression models were used to analyze the independent effects of green tea, coffee, and black tea consumption on the risk of developing dementia or MCI so that the lowest category (none) served as the reference group. Model was adjusted for sex, age, history of hypertension, diabetes mellitus, and hyperlipidemia, formal education, apolipoprotein E (*ApoE*) phenotype status (*ApoE* E4+ or E4-), smoking status, alcohol consumption, green tea, coffee and/or black tea consumption, physical activities and/or hobbies*P-value < 0.05, **P-value < 0.01

tea, such as EGCG and myricetin, would be candidates to exert preventive effects on cognitive decline (Fig. 4.2).

4.2 Effects of Natural Phenolic Compounds in Cerebral Amyloidosis Models

4.2.1 Studies Using *In Vitro* Models of Cerebral Amyloidosis

Natural phenolic compounds have been commonly reported as having anti-oxidant, anti-inflammatory, and other activities that may exert neuroprotective effects on AD and other dementias. However, the remarkable effects of such compounds on cognitive decline observed in epidemiological studies with older people suggest that they may have more specific effects on pathways involved in the pathophysiology of cerebral amyloidosis and other neurodegenerative disorders such as dementia with Lewy bodies (DLB).

Cerebral parenchymal deposition of the amyloid β-peptide (Aβ) is a central feature of AD. In addition, Aβ deposits in the cerebral vasculature of older subjects and AD, called cerebral amyloid angiopathy (CAA), cause cerebral hemorrhages and other cerebrovascular disorders. As amyloid deposition is considered to be the most upstream event in AD pathogenesis (amyloid cascade hypothesis), the process of Aβ deposition is the main target of drug development in AD. Although α-cleavage of amyloid-β precursor protein (APP) by α-secretase prevents production of Aβ, β- and γ-cleavages of APP by β- and γ-secretases produce Aβ; Aβ peptides subsequently aggregate from monomers to oligomers, protofibrils, and fibrils (Fig. 4.3). Moreover, tau protein is phosphorylated and aggregates forming intracellular neurofibrillary tangles composed of paired helical filaments. Finally, synaptic dysfunction and neuronal death occur. In DLB as well as Parkinson's

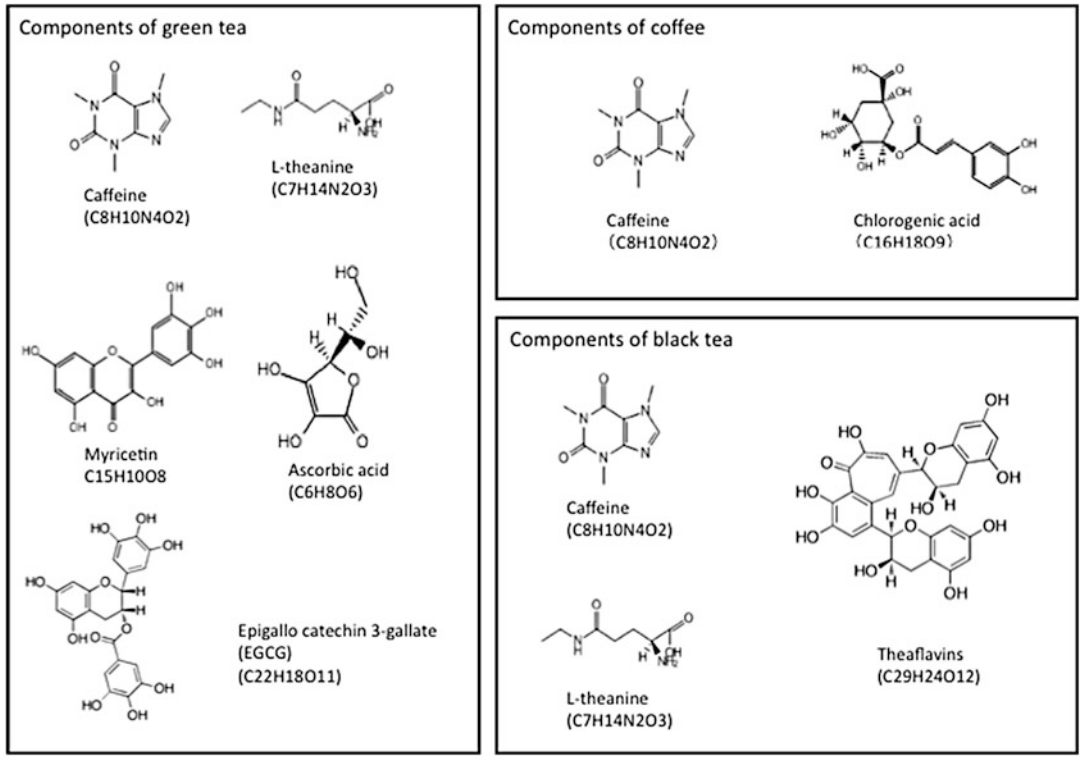


Fig. 4.2 Components of green tea, coffee, and black tea that may be implicated in preventive effects on cognitive decline

disease (PD), α-synuclein (αS) is aggregated in neuronal cell bodies and neurites (Lewy bodies and neurites) in the brain (α-synucleinopathies). Widespread tau aggregation is found in other neurodegenerative dementias than AD (non-AD tauopathies), such as Pick's disease, argyrophilic grain disease, and senile dementia of the neurofibrillary tangle type.

Recent studies have reported that natural phenolic compounds have the following specific actions: modulation of the processing of APP (Levites et al. 2003; Rezai-Zadeh et al. 2005; Obregon et al. 2006; Chakraborty et al. 2011; Kostomoiri et al. 2013; Yoshida et al. 2014; Zhang et al. 2013), inhibition of Aβ aggregation and remodeling and destabilization of aggregates (Ono et al. 2002, 2003, 2004a, b, 2005, 2008, 2012; Yang et al. 2005; Bastianetto et al. 2006; Rivière et al. 2007, 2009; Ehrnhoefer et al. 2008; Shoval et al. 2008; Wang et al. 2008, 2014; Bieschke et al. 2010; Grelle et al. 2011; Thapa et al. 2011; Rigacci et al. 2011; Hirohata et al. 2012; Ge et al. 2012; Cheng et al. 2013; Sinha et al. 2012; Rushworth et al. 2013; Palhano et al. 2013; Zhang et al. 2013; Ho et al. 2013; Cui et al. 2013; Richard et al. 2013; da Silva Bittencourt et al. 2014), promotion of Aβ degradation/clearance (Marambaud et al. 2005; Vingtdeux et al. 2010), alleviation of Aβ-induced oxidative stress/toxicity/synaptic dysfunction (Ono et al. 2003; Savaskan et al. 2003; Sultana et al. 2005; Bastianetto et al. 2006; Joshi et al. 2006; Feng et al. 2009, 2013; Bieschke et al. 2010; Choi et al. 2010; He et al. 2011; Fuentealba et al. 2011, 2012; Grelle et al. 2011; Rushworth et al. 2013; Ho et al. 2013; Wong et al. 2013; Cimini et al. 2013; Camilleri et al. 2013; da Silva Bittencourt et al. 2014), inhibition of αS aggregation (Ono and Yamada 2006; Masuda et al. 2006, 2009; Ehrnhoefer et al. 2008; Bieschke et al. 2010; Grelle et al. 2011; Marchiani et al. 2013), detoxification

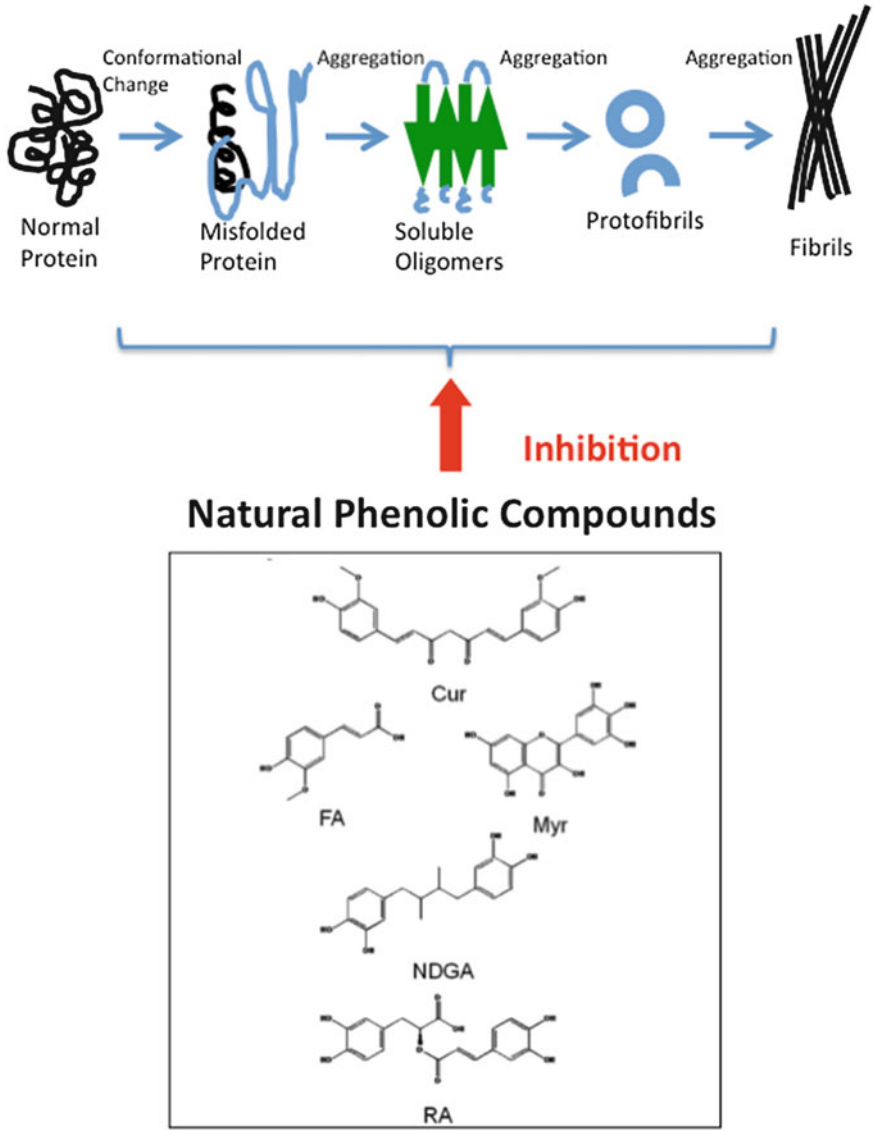


Fig. 4.3 A pathway of protein aggregation for the amyloid-β peptide (Aβ) and α-synuclein protein. The same five phenolic compounds with inhibitory effects on Aβ aggregation in our *in vitro* studies were used for our *in vivo* studies with an animal model. *Cur* curcumin, *FA* ferulic acid, *Myr* myricetin, *NDGA* nordihydroguaiaretic acid, *RA* rosmarinic acid

of αS aggregates (Bieschke et al. 2010; Grelle et al. 2011; Caruana et al. 2012; Marchiani et al. 2013; Lorenzen et al. 2014), and inhibition of tau phosphorylation and aggregation (Taniguchi et al. 2005; Ho et al. 2009b; Ksiezak-Reding et al. 2012; Patil et al. 2013; Yao et al. 2013). Such effects have been reported in various phenolic compounds: flavones such as baicalein (Caruana et al. 2012), flavonols such as Myr, quercetin, and morin (Ono et al. 2003, 2006a, 2012; Masuda et al. 2006; Chakraborty et al. 2011; Caruana et al. 2012; Ho et al. 2013), isoflavones such as glycitein and genistein (Hirohata et al. 2012), flavanols such as EGCG and theaflavins (Ono et al. 2003, 2006a; Levites et al. 2003; Rezai-Zadeh et al. 2005; Obregon et al. 2006;

Bastianetto et al. 2006; Ehrnhoefer et al. 2008; Bieschke et al. 2010; He et al. 2011; Grelle et al. 2011; Cheng et al. 2013; Sinha et al. 2012; Rushworth et al. 2013; Palhano et al. 2013; Zhang et al. 2013; Lorenzen et al. 2014), stilbenes such as resveratrol, nordihydroguaiaretic acid (NDGA), piceid, and viniferin (Ono et al. 2002, 2003, 2006a, 2012; Savaskan et al. 2003; Marambaud et al. 2005; Rivière et al. 2007, 2009; Gauci et al. 2011; Capiralla et al. 2012; Ge et al. 2012; Feng et al. 2013; Vingtdeux et al. 2010; Caruana et al. 2012; Rushworth et al. 2013; Richard et al. 2013), phenolic acids such as rosmarinic acid (RA), tannic acids (TA), ferulic acid (FA), ellagic acid, and gallic acid (Ono et al. 2003, 2004b, 2005, 2006a, 2012; Sultana et al. 2005; Joshi et al. 2006; Feng et al. 2009; Cui et al. 2013; Zhang et al. 2013; Yao et al. 2013; Yoshida et al. 2014), curcuminoids such as curcumin (Cur) (Ono et al. 2004b, 2006a, 2012; Yang et al. 2005; Shoval et al. 2008; Marchiani et al. 2013; Patil et al. 2013), secoiridoids such as oleuropein (Rigacci et al. 2011; Kostomoiri et al. 2013), and others (Thapa et al. 2011). In addition, extracts of phenolic compounds of natural products have been used for studies, including extracts of grape seeds, wine, berries, tea, cocoa, guarana, and *Pueraria lobata* (Ono et al. 2008; Wang et al. 2008, 2014; Ho et al. 2009a; Choi et al. 2010; Fuentealba et al. 2011, 2012; Gauci et al. 2011; Caruana et al. 2012; Ksiezak-Reding et al. 2012; Wong et al. 2013; Cimini et al. 2013; da Silva Bittencourt et al. 2014).

To develop therapeutics and preventives for cerebral Aβ amyloidosis (AD and CAA), we investigated whether such natural phenolic compounds with possible anti-dementia/AD effects suggested in the epidemiological studies have anti-aggregation effects on Aβ. We first examined the effects of Myr, morin, quercetin, kaempferol (+)-catechin, (−)-epicatechin, NDGA, Cur, RA, and FA on the formation, extension, and destabilization of Aβ fibrils (fAβ) *in vitro*, using fluorescence spectroscopy with thioflavin T and electron microscopy (Ono et al. 2003, 2004b, 2006b). All examined phenolic compounds dose-dependentlyinhibited formation of fAβ from fresh Aβ(1–40) (Aβ40) and Aβ(1–42) (Aβ42), as well as their extension (Fig. 4.4a–c). Moreover, these polyphenols dose-dependently destabilized preformed fAβ40 and fAβ42. The effective concentrations (EC50) of Myr, morin, quercetin, NDGA, Cur, and RA for the formation, extension and destabilization of fAβ40 and fAβ42 were in the order of 0.1–1 μM. In cell culture experiments, Myr-treated fAβ were less toxic than intact fAβ, as demonstrated by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assays.

We further investigated the effects of Myr, NDGA, FA, Cur, and RA on Aβ oligomerization and a mechanistic basis of the anti-aggregation effects of these compounds (Ono et al. 2012). We revealed that, using the method of photo-induced cross-linking of unmodified proteins (PICUP), these five phenolic compounds dose-dependently inhibited oligomerization of Aβ40 and Aβ42 (Fig. 4.4d). The circular dichroism (CD) spectroscopy studies showed that both Myr and RA stabilized Aβ populations comprising mostly random coil and inhibited statistical coils to β-sheet conversion. However, at the atomic level, a study with nuclear magnetic resonance (NMR) spectroscopy showed that Myr and RA behave differently, in that Myr shows significant binding to monomeric Aβ42 (Fig. 4.5), whereas RA does not bind to the monomer. It is possible that RA could prevent aggregation by binding to non-NMR detectable early-formed oligomers or distinct monomer conformers/structures causing the inhibition of oligomerization (Fig. 4.5).

There has been mounting evidence that Aβ oligomers rather than mature fibrils are toxic and considered to induce the deleterious cascade(s) involved in the pathophysiology of AD [see review (Larson and Lesne 2012)]. We investigated whether these phenolic compounds with anti-oligmerization effects could attenuate toxicity (Ono et al. 2012). Long-term potentiation (LTP) and depression (LTD) are neurophysiological models of neuronal plasticity for memory and learning; using electrophysiological assays for LTP and LTD in hippocampal slices, we found that Myr and RA decreased Aβ oligomer-induced synaptic toxicities. We evaluated the effects of these phenolic compounds on Aβ-oligomer in-

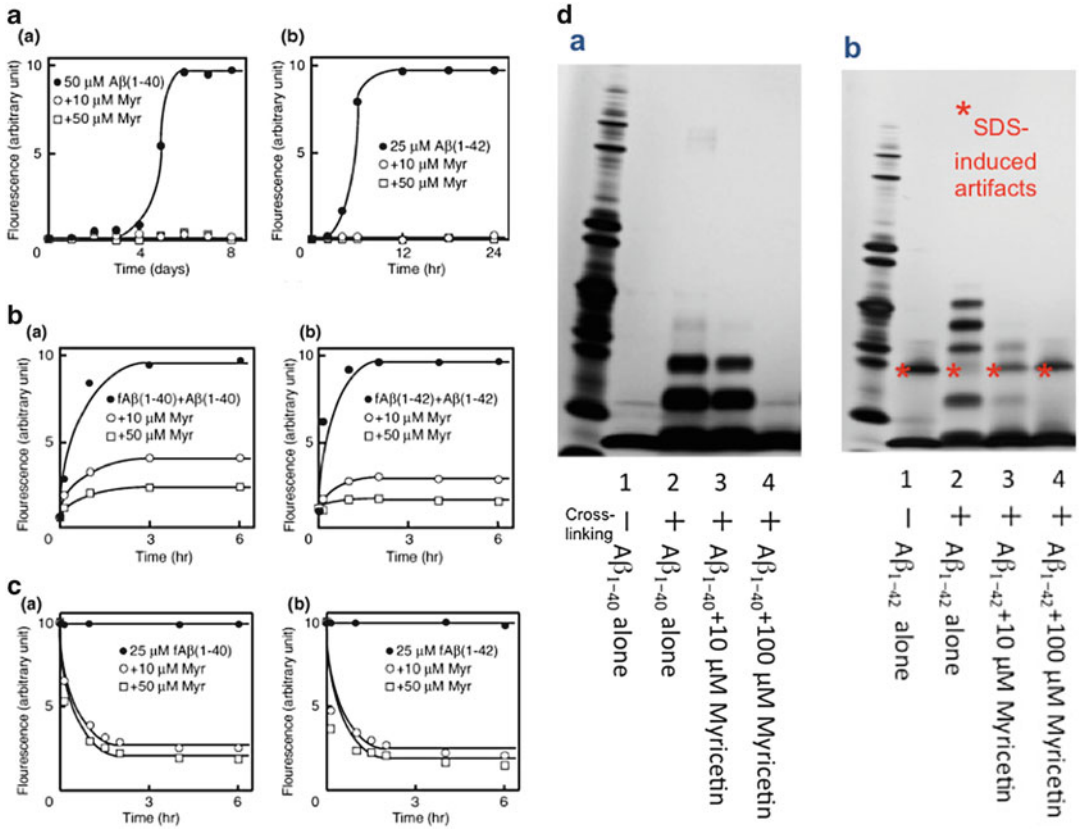


Fig. 4.4 Myricetin inhibits formation of Aβ fibrils (fAβ) from fresh Aβ(1–40) (Aβ40) and Aβ(1–42) (Aβ42) (**A**), extension of fAβ (**B**), destabilized preformed fAβ (**C**), and inhibit oligomerization of Aβ40 and Aβ42 (**D**). The inhibitory and destabilizing effects are also demonstrated with other methods such as electron microscopy and atomic force microscopy (not shown). Details of the experiments were described in the references (Ono et al. 2003, 2012) [**A**, **B**, **C**: thioflavin T; **D**: Photo-induced Cross-linking of Unmodified Proteins (PICUP)]

duced cytotoxicity using MTT assays. Aβ40 and Aβ42 oligomers exhibited cellular toxicity, however, Myr and RA reduced the Aβ oligomer-induced cytotoxicity.

4.2.2 Studies with *In Vivo* Models of Cerebral Amyloidosis

The natural phenolic compounds with *in vitro* anti-amyloidogenic effects have been tested for the effects in *in vivo* models of cerebral amyloidosis. Reductions of amyloid deposition, Aβ oligomer levels, inflammation, or oxidative stress in the brain with attenuation of cognitive deterioration have been reported in transgenic mouse models treated with: Cur (Lim et al. 2001; Yang et al. 2005; Hamaguchi et al. 2009; Ray et al. 2011), EGCG (Rezai-Zadeh et al. 2005, 2008), Myr (Hamaguchi et al. 2009), RA (Hamaguchi et al. 2009), resveratrol (Karuppagounder et al. 2009; Capiralla et al. 2012; Solberg et al. 2014; Porquet et al. 2014), tannic acid (Mori et al. 2012), FA (Mori et al. 2013), rutin (a glycone of quercetin) (Xu et al. 2014), oleuropein (Grossi et al. 2013), hopeahainol A (Zhu et al. 2013), grape seed polyphenolic extract (GSPE) (Wang et al. 2008; Liu et al. 2011), proanthocyanidins of GSPE (Wang et al. 2012), red wine/its polyphenolic contents (Wang et al. 2006; Ho et al. 2009a), anthocyanin-enriched blueberry and blackcurrant

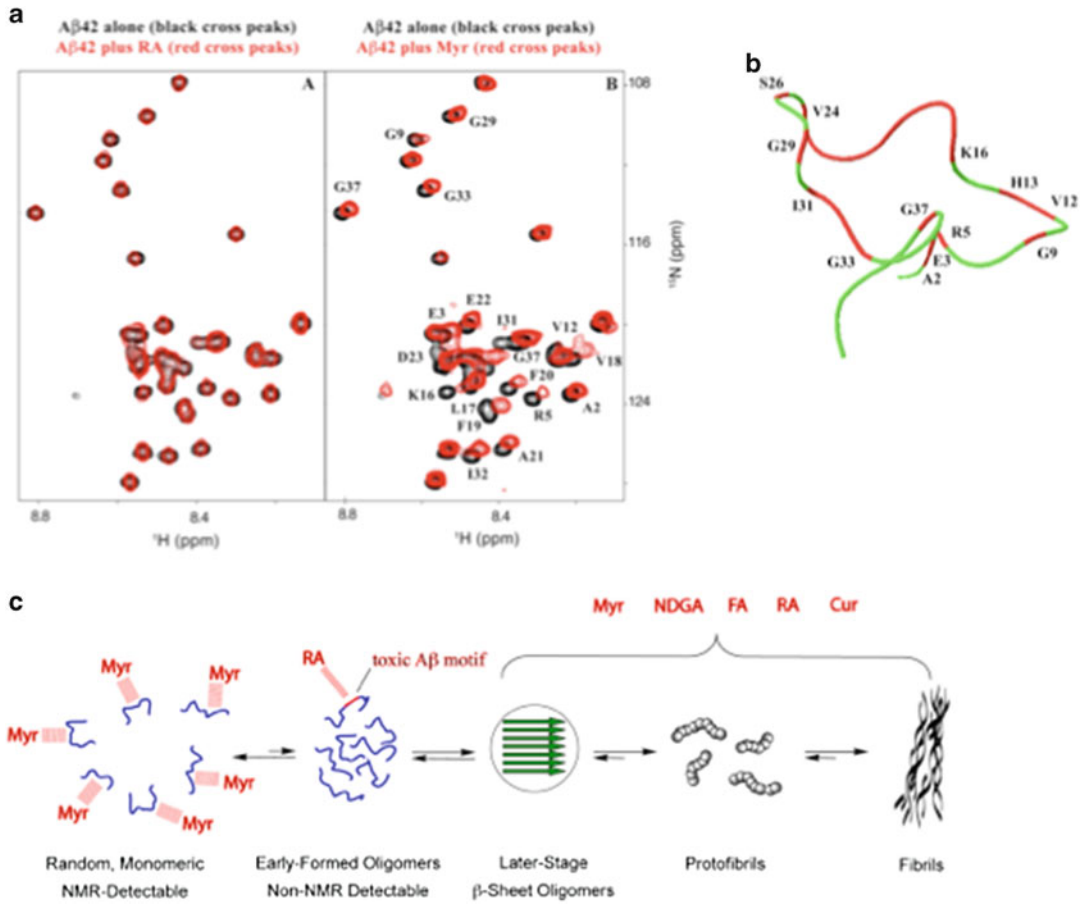


Fig. 4.5 Binding of phenolic compounds to Aβ (**a**). In nuclear magnetic resonance (NMR) spectroscopy studies, myricetin (Myr) shows NH chemical shift movements indicative of binding (*right*), but rosmarinic acid (RA) does not (*left*) (**b**). A representative structural model of Aβ42 that shows binding locations with Myr (indicated by *red* color) (**c**). The summary of our studies for mechanism of polyphenolic inhibition of Aβ aggregation. The phenolic compounds [Myr, RA, nordihydroguaiaretic acid (NDGA), ferulic acid (FA), and curcumin (Cur) (see Fig. 4.3)] exert inhibitory effects through different binding to Aβ. Details of the studies were described in the reference (Ono et al. 2012)

extracts (Vepsäläinen et al. 2013), pomegranate juice containing high levels of polyphenols (Hartman et al. 2006), and a natural diet rich in polyphenols and polyunsaturated fatty acids (LMN diet) (Fernández-Fernández et al. 2012). Other models include an Aβ-infused rat AD model treated with Cur (Hoppe et al. 2013), and a transgenic *Caenohabditis elegans* model of Aβ amyloidosis treated with quercetin (Regitz et al. 2014) and oleuropein (Diomede et al. 2013; Grossi et al. 2014). Furthermore, attenuation of neuropathology was reported in a tau transgenic mouse model of tauopathy treated with GSPE (Wang et al. 2010; Santa-Maria et al. 2012). In addition, EGCG prevented the accumulation of αS in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice, a model of α-synucleinopathy (Mandel et al. 2004).

We focused on the natural phenolic compounds that exerted anti-Aβ aggregation effects in our *in vitro* studies as described above, and systematically investigated whether these five phenolic compounds (Cur, FA, Myr, NDGA, and RA) (Fig. 4.3) also have *in vivo* effects in APP transgenic mice (Tg2576) that show

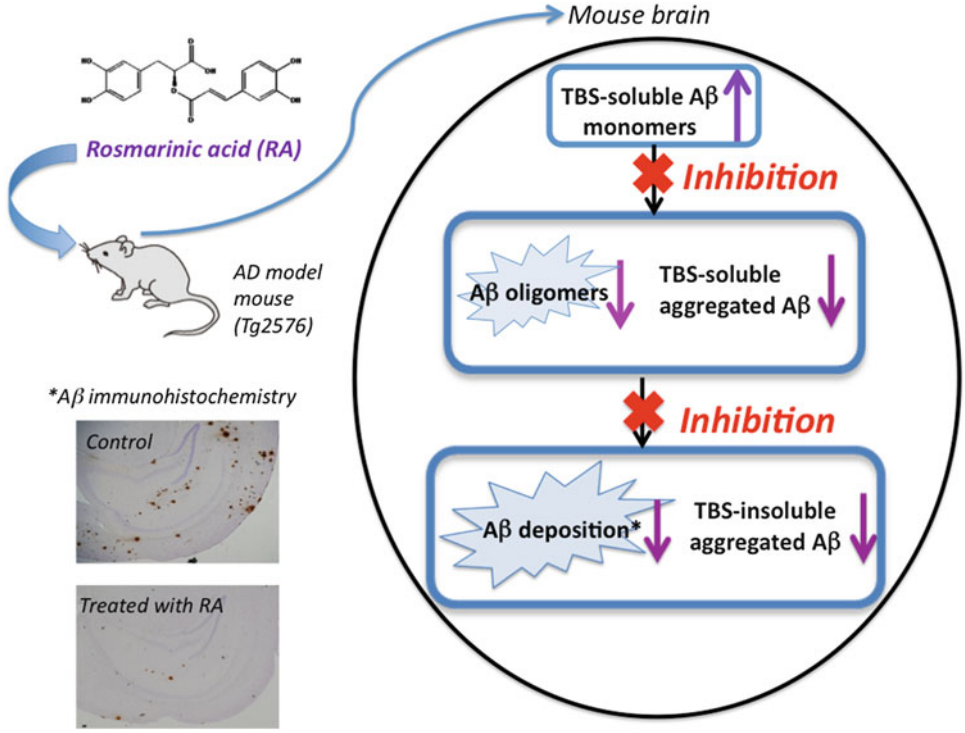


Fig. 4.6 Treatment of Alzheimer's disease (AD) model mice (Tg2576) with rosmarinic acid (RA) showed reductions of both soluble aggregated Aβ, such as Aβ oligomers, and insoluble aggregated Aβ, and an increase of Aβ monomers in the brain. These findings indicate that RA inhibits both the steps from Aβ monomers to oligomers and from oligomers to fibrils. Details of the *in vivo* study on the effects of treatment with diets of the natural phenolic compounds were described in the reference (Hamaguchi et al. 2009)

cerebral Aβ amyloidosis including parenchymal and vascular amyloid deposition (Hamaguchi et al. 2009). Mice were fed Cur, FA, Myr, NDGA, or RA for 10 months from the age of 5 months. Immunohistochemical analysis, in both the NDGA- and RA-treated groups, revealed that Aβ deposition was significantly decreased in the brain ($p < 0.05$). In the RA-treated group, the level of soluble Aβ monomers was increased ($p < 0.01$), while that of oligomers, as probed with the A11 antibody (A11-positive oligomers), was decreased ($p < 0.001$) (Fig. 4.6). However, in the NDGA-treated group, the abundance of A11-positive oligomers was increased ($p < 0.05$) without any change in the levels of soluble or insoluble Aβ. In the Cur- and Myr-treated groups, changes in the Aβ profile were similar to those in the RA-treated group, but Aβ plaque deposition was not significantly decreased. In the FA-treated group, there was no significant difference in the Aβ profile. These results showed that oral administration of the natural phenolic compounds influenced AD pathology and Aβ monomer/oligomer/fibril deposition levels in the brain by differentially modulating Aβ aggregation pathways *in vivo*. From our results, RA appeared to be the best compound, because it was found to inhibit both steps from monomers to soluble oligomers, and from soluble oligomers to insoluble aggregated Aβ deposition (Fig. 4.6). Cur and Myr also seemed effective because they significantly decreased soluble oligomer levels, although the reduction of Aβ deposition did not reach significant levels. FA showed no significant effect. NDGA would be inappropriate, because it significantly increased soluble Aβ oligomer levels, which would suggest that it inhibited only the step from soluble oligomers to

insoluble aggregated Aβ deposition, resulting in an increase of potentially toxic soluble oligomers.

4.3 Clinical Trials with Natural Phenolic Compounds for Alzheimer's Disease

For clinical use, several phenolic compounds have been investigated or are under current investigation in clinical trials. Concerning Cur, two clinical trials in AD have been published. In a double-blind, placebo-controlled, randomized, 6-month trial of Cur with 34 AD patients in Hong Kong, 4 g, 1 g (plus 3 g placebo), or 0 g (plus 4 g placebo) of Cur in addition to 120 mg ginkgo leaf extract were orally administered once daily, and showed no significant difference in changes in MMSE or plasma Aβ40 levels between 0 and 6 months (Baum et al. 2008). Cur showed no significant side effects in this pilot study (Baum et al. 2008). In another double blind, placebo-controlled, randomized, 24-week trial of Cur in California, 34 patients with AD daily received placebo, 2 g, or 4 g of Curcumin C3 Complex® (Ringman et al. 2012). There were no differences between treatment groups in clinical or biomarker efficiency measures including the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog), levels of Aβ40 and Aβ42 in plasma, levels of Aβ42 and total and phosphorylated tau in cerebrospinal fluid (CSF) (Ringman et al. 2012). For adverse effects, Cur was largely well–tolerated, however, three subjects in the Cur group withdrew due to gastrointestinal symptoms (Ringman et al. 2012). Pharmacokinetic results for Cur and its metabolites suggested limited bioavailability of this compound; levels of native Cur were undetectable in the CSF (Ringman et al. 2012). These published data failed to demonstrate clinical or biomarker evidence of efficacy of a half-year oral Cur intake. Further studies are necessary with a larger number of patients, a longer duration of treatment, and better Cur preparations with higher bioavailability and penetration to the brain.

The website of the U.S. National Institute of Health (NIH) (ClinicalTrials.gov) reports that clinical studies with Cur for subjects at the stage of MCI are ongoing. In a double blind, randomized interventional study at UCLA, subjects with MCI or age-associated cognitive impairment are recruited and receive Cur (Theracurmin® 180 mg/day) or placebo; outcome measures include cognitive testing, amyloid PET, and inflammatory markers. Effects of Cur (800 mg) and yoga in subjects with MCI are investigated in a double blind, randomized trial using interventions by Cur or placebo, and aerobic or non-aerobic yoga.

Regarding resveratrol, to our knowledge, there have been no publications of clinical trials for AD or dementia. The NIH website reports that three clinical trials of resveratrol for AD or MCI are active or completed (ClinicalTrials.gov). A double blind, placebo-controlled, randomized, multicenter study operated by the Alzheimer's Disease Cooperative Study in the U.S. is ongoing; it is scheduled that 120 subjects with AD will be enrolled and receive resveratrol (500 mg to 2 g/day by mouth) or placebo for 52 weeks, and CSF markers and MRI as well as safety and tolerability are primary outcome measures. A single-center, multi-site, randomized, double blind, placebo-controlled 12-month trial of liquid resveratrol with glucose and malate to slow the progression of AD in New York has been completed with enrollment of 27 AD subjects, but no results are posted. To test enhancement of memory functions in subjects with MCI by dietary interventions and in combination with exercise and cognitive training, a double blind, placebo-controlled, randomized trial in Germany is ongoing with multiple arms that include a group of resveratrol supplementation.

Clinical trials with EGCG for AD or Down syndrome are also posted on the NIH website (ClinicalTrials.gov). A double-blind, placebo-controlled, randomized, 18-month trial of EGCG in early or mild AD is ongoing in Germany; it is planned that 50 patients will be recruited and receive EGCG (200–800 mg) or placebo added to donepezil with evaluation of cognitive functions. Older subjects with Down syndrome show progression of AD-like lesions in the brain. To test improvement of cognitive performance and deceleration of AD-like progression in

Down syndrome by EGCG, a double-blind, placebo-controlled, randomized study is ongoing in Spain; the participants receive a daily oral dose containing 9 mg/kg of EGCG or placebo, and changes in cognitive functions and amyloid markers are primary outcome measures.

In addition, two clinical trials of isoflavones (including genistein) in AD and two interventional trials of pomegranate polyphenol extract or juice in non-demented subjects are ongoing (ClinicalTrials.gov).

Our group started clinical studies with RA, based on the results of our *in vitro* and *in vivo* studies with models of Aβ amyloidosis (Ono et al. 2004b, 2012; Hamaguchi et al. 2009). First, we completed a double-blind, placebo-controlled, randomized trial in healthy individuals to reveal pharmacokinetics, safety, and tolerability of RA. Next, we are conducting a double-blind, placebo-controlled, randomized trial of RA for mild AD with investigations of cognitive functions and biomarkers including amyloid PET and CSF markers.

Targets of future clinical trials with natural phenolic compounds will extend to other cerebral amyloidoses or protein aggregation disorders than AD, including CAA, PD, DLB, and non-AD tauopathies.

4.4 Conclusions

Epidemiological studies suggest an association of diets rich in phenolic compounds or polyphenols (Mediterranean diet, red wine, green tea, etc.) with reduction of risk of dementia or AD. In addition to the general beneficial effects of these compounds such as anti-oxidant and anti-inflammatory properties, experimental studies indicate that natural polyphenols have specific effects on pathways involved in the pathophysiology of cerebral amyloidosis; the effects include modulation of APP processing, inhibition of Aβ aggregation and destabilization of aggregates, promotion of Aβ degradation/clearance, alleviation of Aβ-induced oxidative stress, leading to reductions of amyloid deposition, Aβ oligomer levels and inflammation in the brain, with attenuation of cognitive deterioration in treated animal models. For clinical use, several phenolic compounds are under investigation by clinical trials for AD or MCI, although no compounds have been yet proved to have certain therapeutic or preventive effects so far. Further clinical trials and preventive interventions of these phenolic compounds with efforts to improve oral bioavailability and brain penetration are necessary to establish their efficacy in AD and other human cerebral amyloidoses.

Acknowledgements This work is supported in part by a Grain-in-Aid Scientific Research (MY, KO, TH, MN-S) and a grant from the Hokuriku Innovation Cluster for Health Science (MY) from the Science of the Ministry of Education, Culture, Sports, Science and Technology of Japan, and a Grant for Health and Labour Sciences Research (the Amyloidosis Research Committee) from the Ministry of Health, Labour, and Welfare, Japan (MY). The authors thank Ms. Etsuko Tsujiguchi for her excellent secretarial assistance.

References

Arntzen KA, Schirmer H, Wilsgaard T et al (2010) Moderate wine consumption is associated with better cognitive test results: a 7 year follow up of 5033 subjects in the Tromsø Study. Acta Neurol Scand 122(S190):23–29

Barone JJ, Roberts HR (1996) Caffeine consumption. Food Chem Toxicol 34(1):119–129

Bastianetto S, Yao ZX, Papadopoulos V et al (2006) Neuroprotective effects of green and black teas and their catechin gallate esters against β-amyloid-induced toxicity. Eur J Neurosci 23(1):55–64

Baum L, Lam CWK, Cheung SK et al (2008) Six-month randomized, placebo-controlled, double-blind, pilot clinical trial of curcumin in patients with Alzheimer disease. J Clin Psychopharmacol 28(1):110–113

Bieschke J, Russ J, Friedrich RP et al (2010) EGCG remodels mature α-synuclein and amyloid-β fibrils and reduces cellular toxicity. Proc Natl Acad Sci U S A 107(17):7710–7715

Camilleri A, Zarb C, Caruana M et al (2013) Mitochondrial membrane permeabilisation by amyloid aggregates and protection by polyphenols. Biochim Biophys Acta 1828(11):2532–2543

Capiralla H, Vingtdeux V, Zhao H et al (2012) Resveratrol mitigates lipopolysaccharide- and Aβ-mediated microglial inflammation by inhibiting the TLR4/NF-κB/STAT signaling cascade. J Neurochem 120(3):461–472

Caruana M, Neuner J, Högen T et al (2012) Polyphenolic compounds are novel protective agents against lipid

membrane damage by α-synuclein aggregates *in vitro*. Biochim Biophys Acta 1818(11):2502–2510

Chakraborty S, Kumar S, Basu S (2011) Conformational transition in the substrate binding domain of β-secretase exploited by NMA and its implication in inhibitor recognition: BACE1-myricetin a case study. Neurochem Int 58(8):914–923

Cheng XR, Hau BY, Veloso AJ et al (2013) Surface plasmon resonance imaging of amyloid-β aggregation kinetics in the presence of epigallocatechin gallate and metals. Anal Chem 85(4):2049–2055

Choi YH, Hong SS, Shin YS et al (2010) Phenolic compounds from *Pueraria lobata* protect PC12 cells against Aβ-induced toxicity. Arch Pharm Res 33(10):1651–1654

Cimini A, Gentile R, D'Angelo B et al (2013) Cocoa powder triggers neuroprotective and preventive effects in a human Alzheimer's disease model by modulating BDNF signaling pathway. J Cell Biochem 114(10):2209–2220

ClinicalTrials.gov https://clinicaltrials.gov. Accessed 18 Aug 2014

Cui L, Zhang Y, Cao H et al (2013) Ferulic acid inhibits the transition of amyloid-β42 monomers to oligomers but accelerates the transition from oligomers to fibrils. J Alzheimers Dis 37(1):19–28

da Silva Bittencourt L, Zeidan-Chulia F, Yatsu FK et al (2014) Guarana (*Paullinia cupana* Mart.) prevents β-amyloid aggregation, generation of advanced glycation-end products (AGEs), and acrolein-induced cytotoxicity on human neuronal-like cells. Phytother Res 28:1615–1624

Dai Q, Borenstein AR, Wu Y et al (2006) Fruit and vegetable juices and Alzheimer's disease: the *Kame* Project. Am J Med 119(9):751–759

Diomede L, Rigacci S, Romeo M et al (2013) Oleuropein aglycone protects transgenic *C. elegans* strains expressing Aβ42 by reducing plaque load and motor deficit. PLoS One 8(3):e58893

Ehrnhoefer DE, Bieschke J, Boeddrich A et al (2008) EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers. Nat Struct Mol Biol 15(6):558–566

Engelhart MJ, Geerlings MI, Ruitenberg A et al (2002) Dietary intake of antioxidants and risk of Alzheimer disease. JAMA 287(24):3223–3229

Eskelinen MH, Ngandu T, Tuomilehto J et al (2009) Midlife coffee and tea drinking and the risk of late-life dementia: a population-based CAIDE study. J Alzheimers Dis 16(1):85–91

Féart C, Samieri C, Rondeau V et al (2009) Adherence to a Mediterranean diet, cognitive decline, and risk of dementia. JAMA 302(6):638–648

Feng Y, Yang SG, Du X et al (2009) Ellagic acid promotes Aβ42 fibrillization and inhibits Aβ42-induced neurotoxicity. Biochem Biophys Res Commun 390(4):1250–1254

Feng X, Liang N, Zhu D et al (2013) Resveratrol inhibits β-amyloid-induced neuronal apoptosis through regulation of SIRT1-ROCK1 signaling pathway. PLoS One 8(3):e59888

Fernández-Fernández L, Comes G, Bolea I et al (2012) LMN diet, rich in polyphenols and polyunsaturated fatty acids, improves mouse cognitive decline associated with aging and Alzheimer's disease. Behav Brain Res 228(2):261–271

Fuentealba J, Dibarrart AJ, Fuentes-Fuentes MC et al (2011) Synaptic failure and adenosine triphosphate imbalance induced by amyloid-β aggregates are prevented by blueberry-enriched polyphenols extract. J Neurosci Res 89(9):1499–1508

Fuentealba J, Dibarrart A, Saez-Orellana F et al (2012) Synaptic silencing and plasma membrane dyshomeostasis induced by amyloid-β peptide are prevented by Aristotelia chilensis enriched extract. J Alzheimers Dis 31(4):879–889

Ganguli M, Chandra V, Kamboh MI et al (2000) Apolipoprotein E polymorphism and Alzheimer disease: the Indo-US Cross-National Dementia Study. Arch Neurol 57(6):824–830

Gauci AJ, Caruana M, Giese A et al (2011) Identification of polyphenolic compounds and black tea extract as potent inhibitors of lipid membrane destabilization by $A\beta_{42}$ aggregates. J Alzheimers Dis 27(4): 767–779

Ge JF, Qiao JP, Qi CC et al (2012) The binding of resveratrol to monomer and fibril amyloid beta. Neurochem Int 61(7):1192–1201

Grelle G, Otto A, Lorenz M et al (2011) Black tea theaflavins inhibit formation of toxic amyloid-β and α-synuclein fibrils. Biochemistry 50(49):10624–10636

Grossi C, Rigacci S, Ambrosini S et al (2013) The polyphenol oleuropein aglycone protects TgCRND8 mice against Aβ plaque pathology. PLoS One 8(8):e71702

Grossi C, Ed Dami T, Rigacci S et al (2014) Employing Alzheimer disease animal models for translational research: focus on dietary components. Neurodegener Dis 13(2–3):131–134

Hamaguchi T, Ono K, Murase A et al (2009) Phenolic compounds prevent Alzheimer's pathology through different effects on the amyloid-β aggregation pathway. Am J Pathol 175(6):2557–2565

Hartman RE, Shah A, Fagan AM et al (2006) Pomegranate juice decreases amyloid load and improves behavior in a mouse model of Alzheimer's disease. Neurobiol Dis 24(3):506–515

He Y, Cui J, Lee JC et al (2011) Prolonged exposure of cortical neurons to oligomeric amyloid-β impairs NMDA receptor function via NADPH oxidase-mediated ROS production: protective effect of green tea (−)-epigallocatechin-3-gallate. ASN Neuro 3(1):e00050

Hirohata M, Ono K, Takasaki J et al (2012) Anti-amyloidogenic effects of soybean isoflavones *in vitro*: fluorescence spectroscopy demonstrating direct binding to Aβ monomers, oligomers and fibrils. Biochim Biophys Acta 1822(8):1316–1324

Ho L, Chen LH, Wang J et al (2009a) Heterogeneity in red wine polyphenolic contents differentially influences Alzheimer's disease-type neuropathology and cognitive deterioration. J Alzheimers Dis 16(1):59–72

Ho L, Yemul S, Wang J et al (2009b) Grape seed polyphenolic extract as a potential novel therapeutic agent in tauopathies. J Alzheimers Dis 16(2):433–439

Ho L, Ferruzzi MG, Janle EM et al (2013) Identification of brain-targeted bioactive dietary quercetin-3-O-glucuronide as a novel intervention for Alzheimer's disease. FASEB J 27(2):769–781

Hoppe JB, Coradini K, Frozza RL et al (2013) Free and nanoencapsulated curcumin suppress β-amyloid-induced cognitive impairments in rats: involvement of BDNF and Akt/GSK-3β signaling pathway. Neurobiol Learn Mem 106:134–144

Joshi G, Perluigi M, Sultana R et al (2006) In vivo protection of synaptosomes by ferulic acid ethyl ester (FAEE) from oxidative stress mediated by 2,2-azobis(2-amidino-propane)dihydrochloride (AAPH) or Fe^{2+}/H_2O_2: insight into mechanisms of neuroprotection and relevance to oxidative stress-related neurodegenerative disorders. Neurochem Int 48(4):318–327

Karuppagounder SS, Pinto JT, Xu H et al (2009) Dietary supplementation with resveratrol reduces plaque pathology in a transgenic model of Alzheimer's disease. Neurochem Int 54(2):111–118

Khokhar S, Magnusdottir SG (2002) Total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom. J Agric Food Chem 50(3):565–570

Kostomoiri M, Fragkouli A, Sagnou M et al (2013) Oleuropein, an anti-oxidant polyphenol constituent of olive promotes α-secretase cleavage of the amyloid precursor protein (AβPP). Cell Mol Neurobiol 33(1):147–154

Ksiezak-Reding H, Ho L, Santa-Maria I et al (2012) Ultrastructural alterations of Alzheimer's disease paired helical filaments by grape seed-derived polyphenols. Neurobiol Aging 33(7):1427–1439

Kuriyama S, Hozawa A, Ohmori K et al (2006) Green tea consumption and cognitive function: a cross-sectional study from the Tsurugaya project[1–3]. Am J Clin Nutr 83(2):355–361

Larson ME, Lesne SE (2012) Soluble Aβ oligomer production and toxicity. J Neurochem 120(S1):125–139

Laurin D, Masaki KH, Foley DJ et al (2004) Midlife dietary intake of antioxidants and risk of late-life incident dementia: the Honolulu-Asia Aging Study. Am J Epidemiol 159(10):959–967

Levites Y, Amit T, Mandel S et al (2003) Neuroprotection and neurorescue against Aβ toxicity and PKC-dependent release of nonamyloidogenic soluble precursor protein by green tea polyphenol (−)-epigallocatechin-3-gallate. FASEB J 17(8):952–954

Lim GP, Chu T, Yang F et al (2001) The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. J Neurosci 21(21):8370–8377

Lindsay J, Laurin D, Verreault R et al (2002) Risk factors for Alzheimer's disease: a prospective analysis from the Canadian Study of Health and Aging. Am J Epidemiol 156(5):445–453

Liu P, Kemper LJ, Wang J et al (2011) Grape seed polyphenolic extract specifically decreases aβ*56 in the brains of Tg2576 mice. J Alzheimers Dis 26(4):657–666

Lorenzen N, Nielsen SB, Yoshimura Y et al (2014) How epigallocatechin gallate can inhibit α-Synuclein oligomer toxicity *in vitro*. J Biol Chem 289(31):21299–21310

Luchsinger JA, Tang MX, Siddiqui M et al (2004) Alcohol intake and risk of dementia. J Am Geriatr Soc 52(4):540–546

Mandel S, Maor G, Youdim MBH (2004) Iron and α-synuclein in the substantia nigra of MPTP-treated mice: effect of neuroprotective drugs R-apomorphine and green tea polyphenol (−)-epigallocatechin-3-gallate. J Mol Neurosci 24(3):401–416

Marambaud P, Zhao H, Davies P (2005) Resveratrol promotes clearance of Alzheimer's disease amyloid-β peptides. J Biol Chem 280(45): 37377–37382

Marchiani A, Mammi S, Siligardi G et al (2013) Small molecules interacting with α-synuclein: antiaggregating and cytoprotective properties. Amino Acids 45(2):327–338

Martínez-Lapiscina EH, Clavero P, Toledo E et al (2013) Mediterranean diet improves cognition: the PREDIMED-NAVARRA randomised trial. J Neurol Neurosurg Psychiatry 84(12):1318–1325

Masuda M, Suzuki N, Taniguchi S et al (2006) Small molecule inhibitors of α-synuclein filament assembly. Biochemistry 45(19):6085–6094

Masuda M, Hasegawa M, Nonaka T et al (2009) Inhibition of α-synuclein fibril assembly by small molecules: analysis using epitope-specific antibodies. FEBS Lett 583(4):787–791

Mehlig K, Skoog I, Guo X et al (2008) Alcoholic beverages and incidence of dementia: 34-year follow-up of the prospective population study of women in Göteborg. Am J Epidemiol 167(6):684–691

Mori T, Rezai-Zadeh K, Koyama N et al (2012) Tannic acid is a natural β-secretase inhibitor that prevents cognitive impairment and mitigates Alzheimer-like pathology in transgenic mice. J Biol Chem 287(9):6912–6927

Mori T, Koyama N, Guillot-Sestier MV et al (2013) Ferulic acid is a nutraceutical β-secretase modulator that improves behavioral impairment and alzheimer-like pathology in transgenic mice. PLoS One 8(2): e55774

Ng TP, Chiam PC, Lee T et al (2006) Curry consumption and cognitive function in the elderly. Am J Epidemiol 164(9):898–906

Noguchi-Shinohara M, Yuki S, Dohmoto C et al (2014) Consumption of green tea, but not black tea or coffee, is associated with reduced risk of cognitive decline. PLoS One 9(5):e96013

Obregon DF, Rezai-Zadeh K, Bai Y et al (2006) ADAM10 activation is required for green tea (−)-epigallocatechin-3-gallate-induced α-secretase cleavage of amyloid precursor protein. J Biol Chem 281(24):16419–16427

Ogawa K, Tsubono Y, Nishino Y et al (2002) Dietary sources of nutrient consumption in a rural Japanese population. J Epidemiol 12(1):1–8

Ono K, Yamada M (2006) Antioxidant compounds have potent anti-fibrillogenic and fibril-destabilizing effects for α-synuclein fibrils *in vitro*. J Neurochem 97(1):105–115

Ono K, Hasegawa K, Yoshiike Y et al (2002) Nordihydroguaiaretic acid potently breaks down pre-formed Alzheimer's β-amyloid fibrils *in vitro*. J Neurochem 81(3):434–440

Ono K, Yoshiike Y, Takashima A et al (2003) Potent anti-amyloidogenic and fibril-destabilizing effects of polyphenols *in vitro*: implications for the prevention and therapeutics of Alzheimer's disease. J Neurochem 87(1):172–181

Ono K, Hasegawa K, Naiki H et al (2004a) Anti-amyloidogenic activity of tannic acid and its activity to destabilize Alzheimer's β-amyloid fibrils in vitro. Biochim Biophys Acta 1690(3):193–202

Ono K, Hasegawa K, Naiki H et al (2004b) Curcumin has potent anti-amyloidogenic effects for Alzheimer's β-amyloid fibrils in vitro. J Neurosci Res 75(6): 742–750

Ono K, Hirohata M, Yamada M (2005) Ferulic acid destabilizes preformed β-amyloid fibrils in vitro. Biochem Biophys Res Commun 336(2):444–449

Ono K, Hasegawa K, Naiki H et al (2006a) Anti-parkinsonian agents have anti-amyloidogenic activity for Alzheimer's β-amyloid fibrils in vitro. Neurochem Int 48(4):275–285

Ono K, Hirohata M, Yamada M (2006b) α-Lipoic acid exhibits anti-amyloidogenicity for β-amyloid fibrils in vitro. Biochem Biophys Res Commun 341(4):1046–1052

Ono K, Condron MM, Ho L et al (2008) Effects of grape seed-derived polyphenols on amyloid β-protein self-assembly and cytotoxicity. J Biol Chem 283(47):32176–32187

Ono K, Li L, Takamura Y et al (2012) Phenolic compounds prevent amyloid β-protein oligomerization and synaptic dysfunction by site-specific binding. J Biol Chem 287(18):14631–14643

Orgogozo JM, Dartigues JF, Lafont S et al (1997) Wine consumption and dementia in the elderly: a prospective community study in the Bordeaux area. Rev Neurol 153(3):185–192

Palhano FL, Lee J, Grimster NP et al (2013) Toward the molecular mechanism(s) by which EGCG treatment remodels mature amyloid fibrils. J Am Chem Soc 135(20):7503–7510

Patil SP, Tran N, Geekiyanage H et al (2013) Curcumin-induced upregulation of the anti-tau cochaperone BAG2 in primary rat cortical neurons. Neurosci Lett 554:121–125

Peterson J, Dwyer J, Bhagwat S et al (2005) Major flavonoids in dry tea. J Food Compos Anal 18(6):487–501

Porquet D, Griñán-Ferré C, Ferrer I et al (2014) Neuroprotective role of trans-resveratrol in a murine model of familial Alzheimer's disease. J Alzheimers Dis 42:1209–1220

Psaltopoulou T, Sergentanis TN, Panagiotakos DB et al (2013) Mediterranean diet, stroke, cognitive impairment, and depression: a meta-analysis. Ann Neurol 74(4):580–591

Ray B, Bisht S, Maitra A et al (2011) Neuroprotective and neurorescue effects of a novel polymeric nanoparticle formulation of curcumin (NanoCurc^{TM}) in the neuronal cell culture and animal model: implications for Alzheimer's disease. J Alzheimers Dis 23(1):61–77

Regitz C, Marie Dußling L, Wenzel U (2014) Amyloid-beta ($A\beta_{1-42}$)-induced paralysis in Caenorhabditis elegans is inhibited by the polyphenol quercetin through activation of protein degradation pathways. Mol Nutr Food Res 58:1931–1940

Rezai-Zadeh K, Shytle D, Sun N et al (2005) Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. J Neurosci 25(38):8807–8814

Rezai-Zadeh K, Arendash GW, Hou H et al (2008) Green tea epigallocatechin-3-gallate (EGCG) reduces β-amyloid mediated cognitive impairment and modulates tau pathology in Alzheimer transgenic mice. Brain Res 1214:177–187

Richard T, Papastamoulis Y, Waffo-Teguo P et al (2013) 3D NMR structure of a complex between the amyloid beta peptide (1–40) and the polyphenol ε-viniferin glucoside: implications in Alzheimer's disease. Biochim Biophys Acta 1830(11):5068–5074

Rigacci S, Guidotti V, Bucciantini M et al (2011) Aβ(1–42) aggregates into non-toxic amyloid assemblies in the presence of the natural polyphenol oleuropein aglycon. Curr Alzheimer Res 8(8):841–852

Ringman JM, Frautschy SA, Teng E et al (2012) Oral curcumin for Alzheimer's disease: tolerability and efficacy in a 24-week randomized, double blind, placebo-controlled study. Alzheimers Res Ther 4(5):43

Ritchie K, Carrière I, de Mendonça A et al (2007) The neuroprotective effects of caffeine: a prospective population study (the Three City Study). Neurology 69(6):536–545

Rivière C, Richard T, Quentin L et al (2007) Inhibitory activity of stilbenes on Alzheimer's β-amyloid fibrils in vitro. Bioorg Med Chem 15(2):1160–1167

Rivière C, Delaunay JC, Immel F et al (2009) The polyphenol piceid destabilizes preformed amyloid fibrils and oligomers in vitro: hypothesis on possible molecular mechanisms. Neurochem Res 34(6):1120–1128

Rushworth JV, Griffiths HH, Watt NT et al (2013) Prion protein-mediated toxicity of amyloid-β oligomers requires lipid rafts and the transmembrane LRP1. J Biol Chem 288(13):8935–8951

Santa-Maria I, Diaz-Ruiz C, Ksiezak-Reding H et al (2012) GSPE interferes with tau aggregation in vivo: implication for treating tauopathy. Neurobiol Aging 33(9):2072–2081

Savaskan E, Olivieri G, Meier F et al (2003) Red wine ingredient resveratrol protects from β-amyloid neurotoxicity. Gerontology 49(6):380–383

Scarmeas N, Stern Y, Tang M et al (2006) Mediterranean diet and risk for Alzheimer's disease. Ann Neurol 59(6):912–921

Scarmeas N, Stern Y, Mayeux R et al (2009) Mediterranean diet and mild cognitive impairment. Arch Neurol 66(2):216–225

Shoval H, Weiner L, Gazit E et al (2008) Polyphenol-induced dissociation of various amyloid fibrils results in a methionine-independent formation of ROS. Biochim Biophys Acta 1784(11):1570–1577

Singh B, Parsaik AK, Mielke MM et al (2014) Association of Mediterranean diet with mild cognitive impairment and Alzheimer's disease: a systematic review and meta-analysis. J Alzheimers Dis 39(2): 271–282

Sinha S, Du Z, Maiti P et al (2012) Comparison of three amyloid assembly inhibitors: the sugar scyllo-inositol, the polyphenol epigallocatechin gallate, and the molecular tweezer CLR01. ACS Chem Neurosci 3(6):451–458

Solberg NO, Chamberlin R, Vigil JR et al (2014) Optical and SPION-enhanced MR imaging shows that trans-stilbene inhibitors of NF-κB concomitantly lower Alzheimer's disease plaque formation and microglial activation in AβPP/PS-1 transgenic mouse brain. J Alzheimers Dis 40(1):191–212

Solfrizzi V, D'Introno A, Colacicco AM et al (2007) Alcohol consumption, mild cognitive impairment, and progression to dementia. Neurology 68(21): 1790–1799

Sultana R, Ravagna A, Mohmmad-Abdul H et al (2005) Ferulic acid ethyl ester protects neurons against amyloid β- peptide(1–42)-induced oxidative stress and neurotoxicity: relationship to antioxidant activity. J Neurochem 92(4):749–758

Taniguchi S, Suzuki N, Masuda M et al (2005) Inhibition of heparin-induced tau filament formation by phenothiazines, polyphenols, and porphyrins. J Biol Chem 280(9):7614–7623

Thapa A, Woo ER, Chi E et al (2011) Biflavonoids are superior to monoflavonoids in inhibiting amyloid-β toxicity and fibrillogenesis via accumulation of non-toxic oligomer-like structures. Biochemistry 50(13): 2445–2455

Truelsen T, Thudium D, Grønbaek M (2002) Amount and type of alcohol and risk of dementia: the Copenhagen City Heart Study. Neurology 59(9):1313–1319

van Gelder BM, Buijsse B, Tijhuis M et al (2007) Coffee consumption is inversely associated with cognitive decline in elderly European men: the FINE Study. Eur J Clin Nutr 61(2):226–232

Vepsäläinen S, Koivisto H, Pekkarinen E et al (2013) Anthocyanin-enriched bilberry and blackcurrant extracts modulate amyloid precursor protein processing and alleviate behavioral abnormalities in the APP/PS1 mouse model of Alzheimer's disease. J Nutr Biochem 24(1):360–370

Vingtdeux V, Giliberto L, Zhao H et al (2010) AMP-activated protein kinase signaling activation by resveratrol modulates amyloid-β peptide metabolism. J Biol Chem 285(12):9100–9113

Wang J, Ho L, Zhao Z et al (2006) Moderate consumption of Cabernet Sauvignon attenuates Aβ neuropathology in a mouse model of Alzheimer's disease. FASEB J 20(13):2313–2320

Wang J, Ho L, Zhao W et al (2008) Grape-derived polyphenolics prevent Aβ oligomerization and attenuate cognitive deterioration in a mouse model of Alzheimer's disease. J Neurosci 28(25): 6388–6392

Wang J, Santa-Maria I, Ho L et al (2010) Grape derived polyphenols attenuate tau neuropathology in a mouse model of Alzheimer's disease. J Alzheimers Dis 22(2):653–661

Wang J, Ferruzzi MG, Ho L et al (2012) Brain-targeted proanthocyanidin metabolites for Alzheimer's disease treatment. J Neurosci 32(15):5144–5150

Wang J, Varghese M, Ono K et al (2014) Cocoa extracts reduce oligomerization of amyloid-β: implications for cognitive improvement in Alzheimer's disease. J Alzheimers Dis 41(2):643–650

Wong DY, Musgrave IF, Harvey BS et al (2013) Açaí (Euterpe oleraceae Mart.) berry extract exerts neuroprotective effects against β-amyloid exposure in vitro. Neurosci Lett 556:221–226

Xu PX, Wang SW, Yu XL et al (2014) Rutin improves spatial memory in Alzheimer's disease transgenic mice by reducing Aβ oligomer level and attenuating oxidative stress and neuroinflammation. Behav Brain Res 264:173–180

Yang F, Lim GP, Begum AN et al (2005) Curcumin inhibits formation of amyloid β oligomers and fibrils, binds plaques, and reduces amyloid *in vivo*. J Biol Chem 280(7):5892–5901

Yao J, Gao X, Sun W et al (2013) Molecular hairpin: a possible model for inhibition of tau aggregation by tannic acid. Biochemistry 52(11):1893–1902

Yoshida H, Meng P, Matsumiya T et al (2014) Carnosic acid suppresses the production of amyloid-β 1–42 and 1–43 by inducing an α-secretase TACE/ADAM17 in U373MG human astrocytoma cells. Neurosci Res 79:83–93

Zhang SQ, Sawmiller D, Li S et al (2013a) Octyl gallate markedly promotes anti-amyloidogenic processing of APP through estrogen receptor-mediated ADAM10 activation. PLoS One 8(8), e71913

Zhang T, Zhang J, Derreumaux P et al (2013b) Molecular mechanism of the inhibition of EGCG on the Alzheimer $A\beta_{1-42}$ dimer. J Phys Chem B 117(15):3993–4002

Zhu X, Ye L, Ge H et al (2013) Hopeahainol A attenuates memory deficits by targeting β-amyloid in APP/PS1 transgenic mice. Aging Cell 12(1):85–92

Brain Food for Alzheimer-Free Ageing: Focus on Herbal Medicines

5

Helmut M. Hügel

Abstract

Healthy brain aging and the problems of dementia and Alzheimer's disease (AD) are a global concern. Beyond 60 years of age, most, if not everyone, will experience a decline in cognitive skills, memory capacity and changes in brain structure. Longevity eventually leads to an accumulation of amyloid plaques and/or tau tangles, including some vascular dementia damage. Therefore, lifestyle choices are paramount to leading either a brain-derived or a brain-deprived life. The focus of this review is to critically examine the evidence, impact, influence and mechanisms of natural products as chemopreventive agents which induce therapeutic outcomes that modulate the aggregation process of beta-amyloid (Aβ), providing measureable cognitive benefits in the aging process. Plants can be considered as chemical factories that manufacture huge numbers of diverse bioactive substances, many of which have the potential to provide substantial neuroprotective benefits. Medicinal herbs and health food supplements have been widely used in Asia since over 2,000 years. The phytochemicals utilized in traditional Chinese medicine have demonstrated safety profiles for human consumption. Many herbs with anti-amyloidogenic activity, including those containing polyphenolic constituents such as green tea, turmeric, *Salvia miltiorrhiza*, and *Panax ginseng*, are presented. Also covered in this review are extracts from kitchen spices including cinnamon, ginger, rosemary, sage, salvia herbs, Chinese celery and many others some of which are commonly used in herbal combinations and represent highly

H.M. Hügel (✉)
School of Applied Sciences & Health Innovations
Research Institute, RMIT University, GPO Box 2476,
Melbourne 3001, Australia
e-mail: helmut.hugel@rmit.edu.au

N. Vassallo (ed.), *Natural Compounds as Therapeutic Agents for Amyloidogenic Diseases*,
Advances in Experimental Medicine and Biology 863, DOI 10.1007/978-3-319-18365-7_5

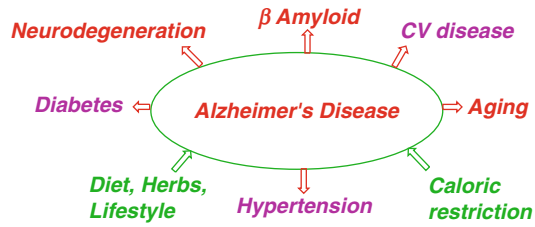
promising therapeutic natural compounds against AD. A number of clinical trials conducted on herbs to counter dementia and AD are discussed.

Keywords
Alzheimer's disease • Dementia • Amyloid-beta • Traditional Chinese medicine (TCM) • Herbal polyphenols

5.1 Beyond the Molecular Frontier – The Threats of Our Age

During the past hundred years, treatments for human diseases have helped raise life expectancy significantly. However, an aging population brings increased burdens and costs to individuals and society from age-related cognitive decline; indeed, the latter has emerged as one of the major health threats and challenges of our age. In another 36 years there will be triple the number of persons 80 years or older, with approximately 50 % of adults over 85 years afflicted with Alzheimer's disease (AD). The total number of new cases of dementia each year worldwide is nearly 7.7 million, which translates to 15 new cases every minute (International 2012). Estimates indicate that between 2 and 10 % of all cases of dementia appear before the age of 65. Advancing age is the highest risk factor for AD, with age-specific prevalence nearly doubling every 5 years beyond the age of 65. The financial estimated worldwide cost of dementia was $604 billion in 2010 (Wimo et al. 2013). Unless we act now, by 2050 the problem will be unmanageable. Recent advances in the biology of aging in model organisms, together with molecular and multidisciplinary studies of neurodegenerative and aging-related disease risks and personal practices (outlined in Scheme 5.1), are beginning to uncover these mechanisms and their potential roles in cognitive decline (Witte et al. 2009; Villeda et al. 2011)

Interrelationships between aging, apolipoprotein E (*APOE*) ε4 allele, oxidative damage, reactive oxygen species (ROS), amyloid metabolism/toxicity and neurodegenerative dysfunctions leading to dementia and AD are highly probable. Nevertheless, the precise mechanisms remain unknown. Ideally, the opportunities for making lifestyle, diet and nutritional choices to enhance human brain and body function is available and practiced by many (Gomez-Pinilla and Tyagi 2013). The theme of positive aging is to be proactive in minimizing/preventing cognitive decline and disease. Dementia and AD research priorities have also advanced from simply considering clinical symptoms. The focus is now more on early detection of the pre-symptomatic phase and the prevalence of early dementia signs, as these are considered to be potential windows of opportunity for successful therapeutic interventions and preventions. For instance, recent research supports mounting evidence implicating dysfunctional lipid metabolism in the pathophysiology of AD indicating that lipid biomarkers have the potential to predict memory impairment at a preclinical stage of AD. Changes in the blood profile of a set of ten lipids critical for proper cell membrane structure and function in elderly persons who showed no signs of cognitive problems, predicted they would go on to develop either mild memory impairment or AD within 2–3 years, with greater than 90 % accuracy (Mapstone et al. 2014).



Scheme 5.1 The interventions and disease risks related to Alzheimer's disease

Humans are able to consume a vast range of foodstuffs. However, the ready availability and low cost of food, and the freedom of being able to eat anything, does not mean that we should maximize eating practices to eat everything (Ulijaszek et al. 2012). The diet-related chronic diseases of modern society are now the single largest cause of death encompassing diabetes, cardiovascular disease, hypertension, obesity and cognitive decline (Scheme 5.1). For foods to promote the health of our aging, physical frailty and mental state, we need to reduce the consumption of processed foods and fatty diets, with negative nutritional attributes such as high-energy refined sugars, saturated fats and high sodium content, whilst increasing affinity and tendency to consume those with positive health attributes including phytochemicals and micronutrient rich foods.

5.2 Herbal Polyphenols – Modulation of Oxidative Stress, Dementia and AD

From our previous analysis of well-designed, randomized double-blind controlled trials on Chinese herbal medicines beneficial for the improvement of cognitive function, we found that neuroprotective benefits of suppression of oxidative stress as the most common feature provided by single herbs or herbal mixtures (May et al. 2009, 2012).

5.2.1 Epigallocatechin-3-Gallate

Oxidative stress may directly initiate neurodegeneration, and herbal antioxidant neuroprotection is considered as a preventative and therapeutic approach (Hugel et al. 2012). Crucially, the scientific evidence confirms that the majority of herbal polyphenolic compounds have a good safety profile, are affordable and are globally readily available to significantly reduce the burden of dementia and AD.

It has been known for at least a decade that polyphenols possess anti-amyloidogenic activity. A diverse range of herbal polyphenolic constituents including tannic acid, quercetin, kaempferol, curcumin, catechin and epicatechin are known to dose-dependently inhibit the formation of amyloid-beta (Aβ) fibrils as well as their elongation. Importantly, polyphenols can bind directly to Aβ or mature aggregates and impair their stability. Epigallocatechin-3-gallate (EGCG), a major component of green tea, significantly inhibits Aβ aggregation and has the ability to remodel large Aβ fibrils into smaller aggregates that are non-toxic (Wang et al. 2010). The gallate functionality in EGCG is critical in facilitating the reduction of Aβ and increasing APP α-proteolysis. Evidence has indicated that EGCG reduces Aβ production in both neuronal and mouse AD models in concert with activation of anti-amyloidogenic amyloid precursor protein (APP) α-processing. An extensive screening of the effect of other gallate-containing phenolic compounds on APP anti-amyloidogenic processing found that long chain gallate esters (Zhang et al. 2013b) such as octyl gallate (OG; 10 mM), a commercial food antioxidant, drastically decreased Aβ generation, in concert with increased APPα-proteolysis in murine neuron-like cells transfected with human wild-type APP or "Swedish" mutant APP. OG markedly increased production of the neuroprotective amino-terminal APP cleavage product, soluble APP-α (sAPPα). OG increases anti-amyloidogenic APPα-secretase processing by activation of ERα/PI3k/Akt signaling and ADAM10. Fish oil has been shown to have a synergistic effect in combination with EGCG, with co-treatment leading to a reduction in Aβ plaque formation and levels of Aβ(1–40) and Aβ(1–42) in AD transgenic Tg2576 mice (Giunta et al. 2010). The potential role of polyphenols in neurodegeneration and the pathogenesis of AD has expanded with discoveries that they can modulate a class of proteins called sirtuins that are involved in longevity and cell survival (Jayasena et al. 2013) (Table 5.1).

EGCG has numerous health-promoting effects (Hugel and Jackson 2012) including anti-cancer, antioxidant, anti-inflammatory, anti-diabetic, anti-aging and in particular its Aβ-sheet disruption (Palhano et al. 2013) capacity

Table 5.1 Anti-amyloidogenic activity of polyphenols and herbal extracts

Polyphenol/herbal extract	Anti-amyloidogenic activity
Investigation of the ability of **EGCG** to inhibit the formation of metal-free or metal-associated Aβ(1–40) aggregates	EGCG interacted with Cu(II)- and Zn(II)-Aβ monomer, dimer species. Formed more compact peptide conformations compared to EGCG-untreated Aβ species; ternary EGCG–metal–Aβ complexes were produced. This illustrates the selective modulation of the anti-amyloidogenic reactivity of EGCG towards metal-Aβ species (Hyung et al. 2013)
Effect of the addition of **EGCG** in drinking water (1.5, 3 mg/kg for 3 weeks) intake in mice	Prevented lipopolysaccharide-induced Aβ production by the inhibition of β-secretase activity, and improved effects on memory deficiency in liposaccharide-induced AD mice models (Lee et al. 2009)
Isothermal titration calorimetry studies on the interactions between **EGCG** and Aβ	EGCG-Aβ binding was enhanced by increasing temperature, salt concentration and at pH values away from the pI of Aβ (Wang et al. 2010)
EGCG encapsulated in nanoparticles	Improved *in vivo* efficacy, doubled bioavailability; improved chemical stability and enhanced its biological activity (Li et al. 2012; Hu et al. 2013; Smith et al. 2010)
Protonation of **EGCG** at low pH	Resulted in aggregation and reduced oral bioavailability of EGCG-dispersed selenium nanoparticles (Wu et al. 2013b)
Modulation of Aβ-induced tau hyperphosphorylation by **curcumin (Cur)** in human neuroblastoma SH-SY5Y cells	Cur inhibits phosphorylation of tau at Thr231 and Ser396 by modulating the phosphatase and tensin homolog (PTEN) PTEN/Akt/GSK-3β pathway. Involves down-regulation of phosphorylation of Akt and of PTEN, a negative regulator of PIP3 induced by Aβ (Huang et al. 2014a)
Effects of **Cur** after 3-month administration to *APPswe/PS1dE9* double transgenic mice, an AD model	Reduced Aβ(1–40) and Aβ(1–42) levels, and aggregation of Aβ-derived diffusible ligands in the mouse hippocampal CA1 area; enhanced expression of γ-secretase; increased expression of β-amyloid-degrading enzymes, including insulin-degrading enzymes and neprilysin (Esatbeyoglu et al. 2012; Wang et al. 2014)
Testing of **Cur**-based fluorescence imaging probes *in vitro* and *in vivo*	Near-infrared fluorescence imaging with the Cur analogue CRANAD-58 revealed interaction with Aβ in mouse brain; CRANAD-17 was capable of inhibiting Aβ42 cross-linking induced by copper (Zhang et al. 2013c)
Targeting of endogenous neural stem cells by **Cur**-encapsulated nanoparticles	Cur nanoparticles: increase neuronal differentiation by activating the Wnt/β-catenin pathway in hippocampal neural stem cells; involved in regulation of neurogenesis; rescued learning and memory impairments in an Aβ-amyloid induced rat model of AD (Tiwari et al. 2014)
Studies on the brain accessibility of **Cur**-lipid-nanoparticles	High affinity for Aβ in post-mortem brains samples of AD patients (Mourtas et al. 2014). Cur-loaded solid lipid nanoparticles showed 30 times higher preferential distribution into the brain (Kakkar et al. 2013)
Anti-amyloidogenic effect of an ethanol extract of ***Magnolia officinalis***: 12.9 % magnolol, 16.5 % honokiol, 16.6 % 4-O–methylhonokiol, plus 42–45 % of other constituents	Administration of 10 mg/kg extract for 3 months inhibited amyloidogenesis, reduced Aβ accumulation via β-secretase 1 (BACE1) inhibition in the brain of Tg2576 mice with memory improving effects (Lee et al. 2012)
2,2′,4′-trihydroxychalcone ***Glycyrrhiza glabra***	Anti-oxidative, anti-tumor, *in vitro* inhibition of BACE1 bioactivity with IC_{50} 2.5 μM, reduced Aβ formation in mice-AD studies (Zhu et al. 2010)

(continued)

Table 5.1 (continued)

Polyphenol/herbal extract	Anti-amyloidogenic activity
Isobavachalcone, bavachinin isolated from ***Psoraleae Fructus***	Contains compounds that inhibit BACE1 (Choi et al. 2008). Isobavachalcone inhibits Aβ oligomerization and fibrillization, bavachinin transforms Aβ into non-toxic aggregates (Chen et al. 2013)
Tenuifolin, a triterpenoid saponin isolated from ***Polygala tenuifolia***	2.0 μg/mL tenuifolin significantly decreased Aβ-secretion from COS-7 cells without altering the ratio of Aβ(1–40) and Aβ(1–42) by BACE1 inhibition (Lv et al. 2009)
Effect of the ***Polygonum multiflorum***extract component 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside (TSG) on the rat Aβ model	Administration of TSG rescued Aβ(1–42) induced impairment in learning and memory, protecting synaptic structures and function; the up-regulation of Src and NR2B may be responsible for the improved learning and anti-AD properties (Zhou et al. 2012)
Effect of ethanol extract of ***Polygonum multiflorum*** in mouse neuroblastoma cells expressing Swedish APP (N2a-SweAPP)	Potent reduction in Aβ production through APP modulation, with the up-regulation of sAPPα and down-regulation of sAPPβ (Liu et al. 2012)
Salvia miltiorrhiza lipophilic constituents: Tanshinone I (TI), Tanshinone (IIA)	Molecular dynamics simulations reveal that TI and TIIA preferentially bind to a hydrophobic β-sheet groove. T1 was better than TIIA for inhibition amyloid–β aggregation; the tanshinones also affected disaggregation of amyloid fibrils, and protection of cultured cells (Wang et al. 2013)
Salvia miltiorrhiza water-soluble constituents: Danshensu and Salvianolic acid B	Protected PC-12 cells by blocking Aβ(25–35) induced Ca^{2+} intake, lactate dehydrogenase release, cell viability decrease and apoptosis (Zhou et al. 2011)
Danshen extract (danshensu 40 mg/kg, protocatechuic aldehyde 149 mg/kg, and salvianolic acid B 50 mg/kg) was administrated intragastrically in rats	From blood and brain microdialysates collected at 15 and 30 min time intervals, danshensu and protocatechuic acid (oxidative metabolite of protocatechuic aldehyde) could be detected in the blood and brain (Zhang et al. 2011)
Examination of **Salvianolic acid B** (Sal B) on human islet amyloid polypeptide (hIAPP) aggregation and phototoxicity	Sal B significantly inhibited the formation of hIAPP amyloid and disaggregated hIAPP fibrils. Cytoprotective effects by Sal B on pancreatic INS-1 cells (Cheng et al. 2013a)

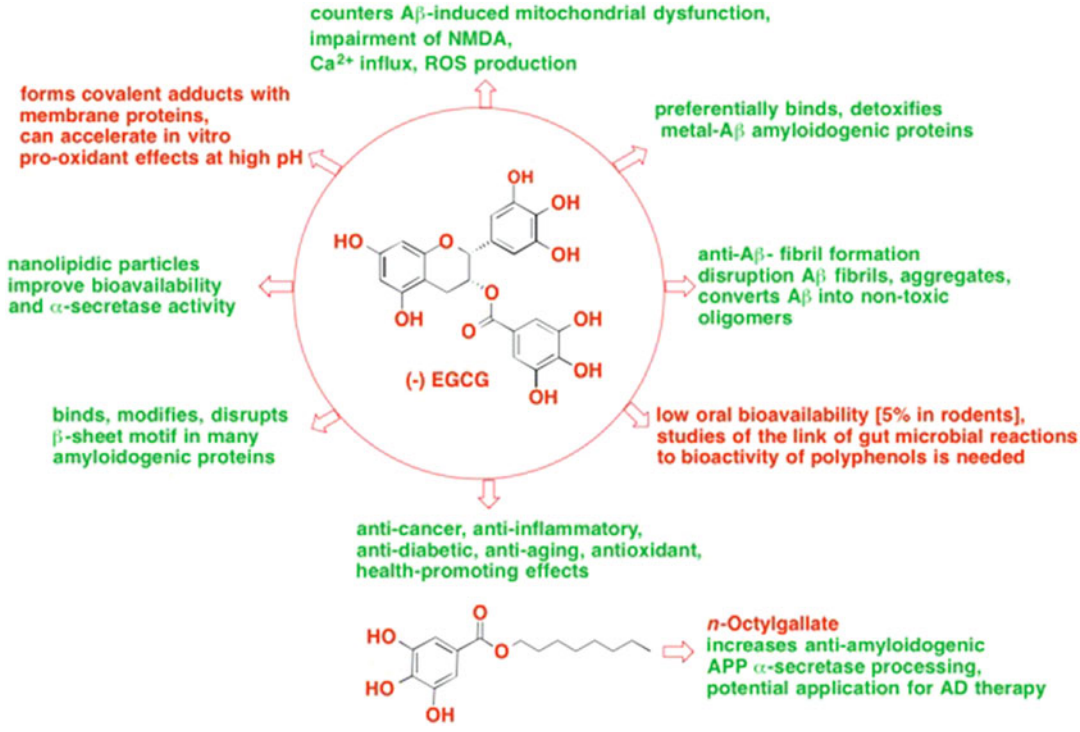
(outlined in Scheme 5.2). The major research challenge concerning the anti-amyloidogenic benefits of polyphenol-containing herbs and foods is to enhance their bioavailability and brain permeability (Schaffer and Halliwell 2012; Singh et al. 2008; Green et al. 2007; Lambert et al. 2006; Smith et al. 2010; van Duynhoven et al. 2011). Furthermore, the bioavailability of polyphenols from dietary input is highly variable between individuals and generally far too low to explain their bioactive antioxidant effects *in vivo* (Lotito and Frei 2006).

5.2.2 Curcumin

Cur is a promising neuroprotective anti-AD natural product that however has poor brain bioavailability with incompletely defined therapeutic mechanisms. Its antioxidant, anti-inflammatory properties have been extensively documented (Esatbeyoglu et al. 2012; Wang et al. 2014). Cur-nanoparticles with improved brain permeability induced adult neurogenesis through activation of the canonical Wnt/β-catenin pathway, and may provide opportunities for treating AD by enhancing a brain self repair mechanism (Zhang et al. 2013c).

5.2.3 *Magnolia officinalis*

The herbal constituents shown in Fig. 5.1 from *Magnolia officinalis* and other members of the *Magnoliaceae* family have diverse therapeutic applications (Lee et al. 2011b). The neolignan



Scheme 5.2 The multiple therapeutic applications of green tea constituent EGCG

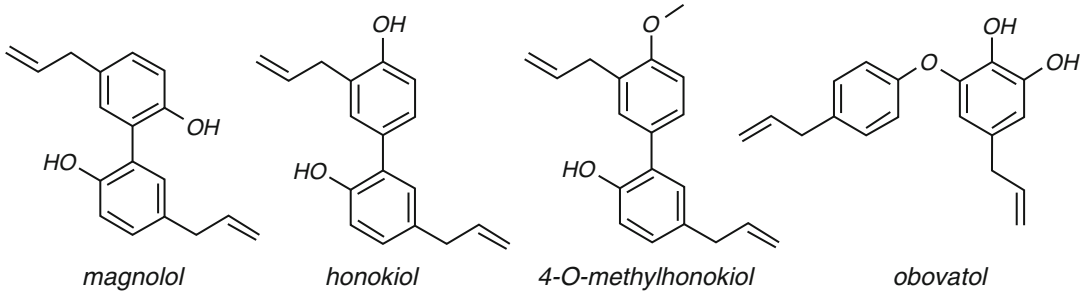
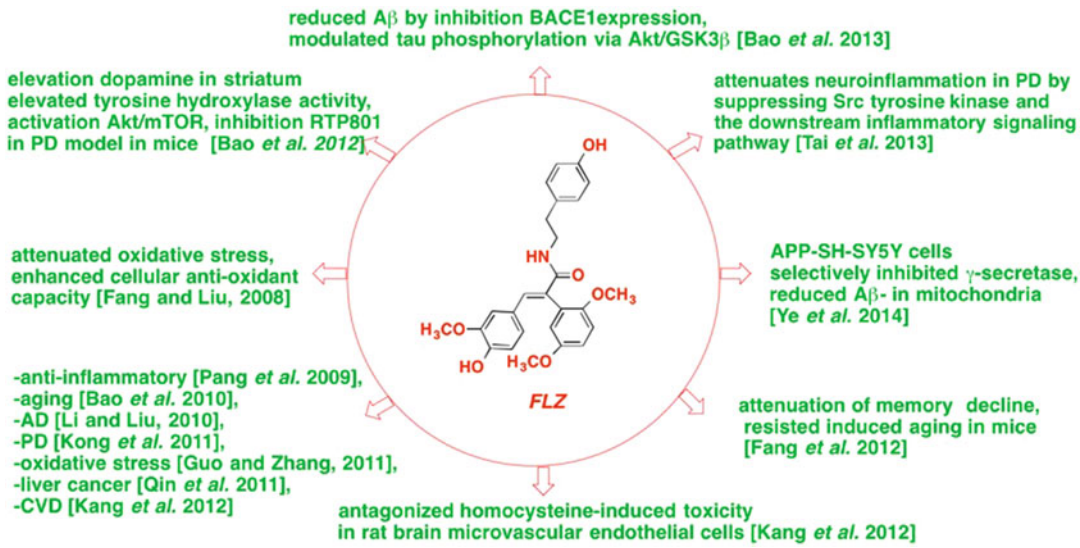


Fig. 5.1 Major bioactive constituents found in *Magnolia officinalis*

4-*O*-methylhonokiol is a potent cannabinoid receptor type-2 (CB2) ligand and has been found to attenuate memory impairment in presenilin 2 mutant mice through reduction of oxidative damage and inactivation of astrocytes and the extracellular signal-regulated kinase (ERK) pathway (Lee et al. 2011a). The various neuroprotective and anti-Alzheimer disease effects reported in rodent models (Lee et al. 2011a) may be mediated via CB2 receptors, providing evidence that the compound should be bioavailable in the brain.

5.2.4 *Annona glabrais* – Squamosamide Derivative (FLZ Compound)

Traditional Chinese medicine makes use of several constituents from the leaves and roots of *Annona glabrais,* including a natural squamosamide. Importantly, the squamosamide derivative FLZ showed enhanced antioxidant activity; in APP-SH-SY5Y expressing cells it selectively inhibited γ-secretase activity without



Scheme 5.3 Anti-amyloidogenic properties of compound FLZ, a squamoside analogue of a constituent from *Annona glabrais*

modulating the Notch pathway (Ye et al. 2014). The many positive anti-amyloidogenic studies suggest FLZ may have therapeutic potential for the treatment of AD (illustrated in Scheme 5.3) (Fang et al. 2012; Kang and Zhang 2012; Pang et al. 2009; Li and Liu 2010; Kong et al. 2011; Qin et al. 2011; Fang and Liu 2008; Bao et al. 2012, 2013; Tai et al. 2013)

5.2.5 *Ginseng*

The available types of ginseng, all belonging to the *Araliaceae* family, are Asian ginseng (*Panax ginseng*), American ginseng (*P. quinquefolus*) and Siberian ginseng (*Eleutherococcus senticosus*). Water extracts of the dried roots and leaves of *Panax ginseng* have been used as a stimulant/tonic, diuretic and digestive aid in traditional Chinese medicine for over 2,000 years. Ginseng phytomedicines are sold as ergogenic supplements to enhance mental and physical performance – reflective of Chinese medicine where body and mind are inseparable – to provide resistance to stress, and to prevent 'exhaustion' and disease. The major active principles of *P. ginseng extracts* are ginsenosides, which are glycosylated derivatives of the triterpene dammarane such as for instance Rg_1. Rg_3 is one of the major constituents of ginseng. The ginsenosides that reduce Aβ levels in animal models and other *in vitro* studies are summarized in Table 5.2.

The diverse constituents and multiple actions of ginseng constituents in the CNS reviewed recently (Kim et al. 2013a) will not be elaborated here. The *in silco* analysis of 12 ginsenosides (see Table 5.2) revealed those with potential interactions with the BACE1 receptor active site essential for enzyme inhibition (Karpagam et al. 2013). Further studies included ADMET screening to find the drug-like ginsenosides with a specific ability to cross blood brain barrier (BBB), and to determine safety/toxicity. Also the BACE1-ginsenosides complexes were further subjected to a molecular dynamics simulation to study their stability and hydrogen bond interactions. Of the 12 ginsenosides, CK, F_1, Rh_1, and Rh_2 were predicted to pass the BBB and ADMET analysis predicted toxic effects for ginsenosides Ro and ginsenoside Rg_1, while Rf showed low oral absorption in human gastrointestinal tract. These results suggest that of the seven ginsenosides demonstrating BACE1

Table 5.2 The anti-AD bioactivities of *P. ginseng* constituents

Panax ginseng AD cognitive effects	Anti-Aβ bioactivities
	Ginsenoside Rg_3 inhibited γ-secretase activity in mouse model AD
	Aβ lowering by modulation/reduction of lipid kinase PI4KIIα activity (Kang et al. 2013)
	Rg_3 enhanced neprilysin (NEP, rate-limiting enzyme in Aβ degradation) gene expression. Caused a reduction in Aβ(1–40) and Aβ(1–42). (Yang et al. 2009)
Fermented red ginseng – ginsenoside Rh_2 neuroprotective effects. Inhibited ischemia reperfusion brain injury in rats (Bae et al. 2004)	
	P. notoginseng modulates protein, gene expression related to α- and β-secretases. Reductions in levels of β-secretase resulting in decline of Aβ generation (Huang et al. 2014b)
Fermented ginseng (FG) ameliorated memory impairment in transgenic mouse model of AD	Brain soluble Aβ(1–42) levels measured from the cerebral cortex of transgenic mice were significantly reduced by the FG extract treatment (Kim et al. 2013b)
	Commercially-available preparations of **ginseng Rg_1**, **Rg_3**, and **RE**, resulted in significant reductions in the amount of Aβ(1–42) detected in the brains of animals after single oral doses of these agents (Chen et al. 2006)
Oral administration of **ginsenoside Rb_1** to mice stressed with acute immobilization; Rb_1 modulated stress effects by attenuating the stress-induced increase in neurosteroids (Lee et al. 2006a)	
Oral administration of **Rg_3** and **Rb_1** to mice stressed with acute immobilization; both lowered levels of the stress-marker putrescine (Lee et al. 2006b)	
Ginsenoside Rg_1 improved learning & memory in rat model of AD (Quan et al. 2013)	**Rg_1** inhibits the transcription and translation of BACE1, suppresses the activity of BACE1, and ultimately attenuates Aβ generation (Chen et al. 2012)
	Rg_1 promoted α-secretase cleavage of APP via estrogenic activity, indicating that it may be useful in the prevention of AD, in particular in postmenopausal females (Shi et al. 2013)
Rg_1, applied to primary cultured cortical neurons, rescued Aβ-mediated mitochondrial dysfunction	May attenuate Aβ-induced neuronal death through the suppression of intracellular mitochondrial oxidative stress (Huang et al. 2012)
Rd attenuated β-amyloid-induced pathological tau phosphorylation	Enhanced the activity of protein phosphatase 2A (PP-2A) involved in tau dephosphorylation (Li et al. 2013a)
In silico approach for discovery of BACE1 inhibitors from ***Panax ginsenosides*** included Rb_1, Rd, Rf, Re, Rg_1, Rg_2, Rg_3, Ro, Rh_1, Rh_2, CK, and F1	Rh_1, Rh_2, CK, F1 passed the criteria of: molecular docking-evaluated interaction with BACE1 receptor proteins, complex stability, H-bond interactions, ADMET for BBB permeability, having no toxicity (Karpagam et al. 2013)
Ginsenoside Rg_5 effect on cognition and beta-amyloid deposition in STZ-induced memory impaired rats	Rg_5 (5, 10 and 20 mg/kg) improved cognitive dysfunction in rats which was related to attenuating neuro-inflammatory responses with decreased brain levels of inflammatory cytokines TNF-α, IL-1β; Congo Red staining and Western blot analysis showed decreased Aβ deposits (Chu et al. 2014)

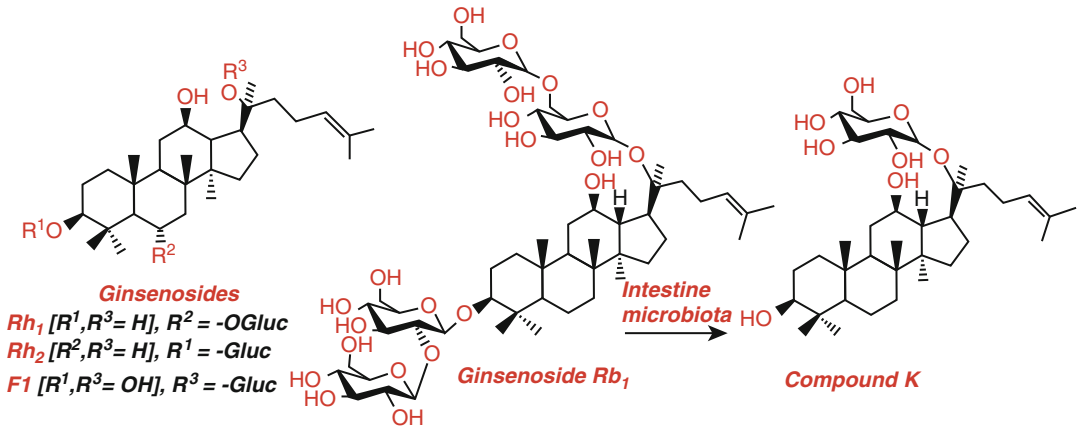


Fig. 5.2 The structures of ginsenosides Rb_1 and its metabolic transformation product K, and those of Rh_1, Rh_2, F_1

inhibition, only the four monoglucosylated ginsenosides CK, Rh_1, Rh_2, and F_1 pass the BBB and possess satisfactory drug-like properties. BACE1 and ginseng inhibitor complex crystal structural data to describe their binding modes would provide an accurate picture of the number and length of hydrophobic and hydrogen bond ginsenoside-enzyme interactions. These two descriptors have reliably predicted the activity of synthetic BACE1 inhibitors (Nastase and Boyd 2012). The wider implications of this research are that the brain-permeation/bioactivity of di- and multi-glycosylated ginsenosides is questionable. Intestinal microbial metabolism (Zhang et al. 2013d) similar to that of Rb_1 shown in Fig. 5.2 may be a pre-requisite for their neuroprotective activity.

5.2.6 Herbal Foods, Formulations and Supplements

L-3-*n*-Butylphthalide (Fig. 5.3) was first extracted from Chinese celery (*Apium graveolens* var. *secalinum*). The chemically prepared compound is used as an anti-hypertensive herbal medicine for the treatment of ischemic stroke, and has therapeutic application for the prevention of vascular dementia by up-regulation of Akt expression in the hippocampus (Huai et al. 2013; Peng et al. 2008, 2012). Potassium 2-(1-hydroxypentyl)-benzoate (dl-PHPB), a precursor to *n*-butylphthalide, has neuroprotective effects on cerebral ischemic, vascular dementia and Aβ-induced animal models by inhibiting oxidative injury, neuronal apoptosis and glial activation. Further research has suggested that dl-PHPB could be an attractive multi-target neuronal protective agent for the treatment of AD (Zhao et al. 2013; Peng et al. 2014). *Z*-ligustilide found in *R. angelica sinensis* promotes the activities of superoxide dismutase and thereby reduces oxidative stress in brain tissues; protects against Aβ-induced neurotoxicity and is a potential therapeutic against vascular dementia (Huang et al. 2008; Kuang et al. 2006; Feng et al. 2012; Xin et al. 2013). An appreciation of the amount of *Z*-ligustilide, the bioactive component in 10 g of herb is detailed in Fig. 5.3. The pharmacokinetics and bioavailability of Z-ligustilide were determined by the systematic investigation in Sprague–Dawley rats. With an extraction efficiency of 62.3 %, 0.93 g *Z*-ligustilide was isolated from 100 g of *R. angelica sinensis*. Therefore, based on animal pharmacokinetic data, with the absolute bioavailability at a 50 mg/kg dose of 75.44 %, a single medicinal use of 10 g of the herb may deliver 43.7 mg of *Z*-ligustilide.

Studies on 27 herbs revealed that some lesser known herbs such as *Curcuma aromatica* and *Zingiber officinale* (ginger) extracts effectively protected cells from Aβ insult, followed by Ginkgo biloba (ginkgo), Polygonatum

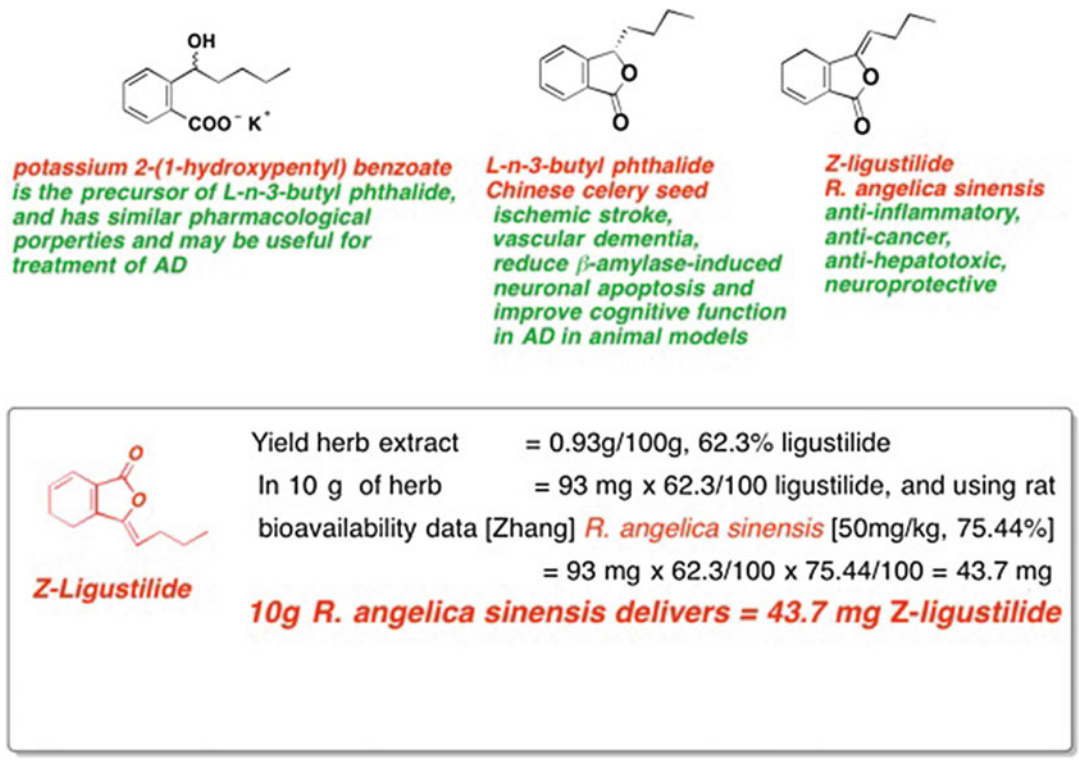


Fig. 5.3 Herbal bioactive compounds and *Z-ligustilide* bioavailability calculations (Zhang et al. 2014)

sp., *Cinnamum cassia* (Chinese cinnamon), *Rheum coreanum* (Korean rhubarb), *Gastrodia elata* (gastrodia), and *Scutellaria baicalensis* (skullcap) (Kim et al. 2007). With regards to herbs, spices and food products that disrupt, destabilize or reverse amyloid aggregation, these have been investigated for their ability: (i) to detour the generation of toxic amyloid precursors (off-pathway); (ii) to prevent the assembly of amyloid oligomers into fibrils; (iii) to inhibit fibril growth and deposition; (iv) to disassemble preformed fibrils; and (v) to promote Aβ clearance. The structures of the active anti-dementia constituents in Chinese herbs most widely used and investigated as potential amyloid inhibitors are presented in Fig. 5.4.

Many herbs are considered to be responsible for multiple beneficial effects such as improving vascular dementia, energy homeostasis, improving mitochondrial antioxidant capacity, and anti-inflammatory neuroprotection. The many and varied constituents in herbs can also enhance the bioavailability and bio-effectiveness of the active constituents and thus have more therapeutic value than individual compounds. Preliminary animal model studies suggest that antioxidants in spearmint and rosemary might be useful in modulating age-associated cognitive decline. Furthermore, rosemary improves local blood circulation, relieves pain, has anticancer activity, and controls blood lipid and anti lipid peroxidation. Carnosic acid, one of the major phenolic constituents of rosemary, is a pro-electrophile specifically activated by the oxidative stress pathological state resulting in its conversion from the hydroquinone to the oxidized quinone form, before it activates the Keap1/Nrf2 pathway leading to gene induction of the antioxidant response element (ARE) and gene products that protect against oxidative stress. A survey of Chinese herbs and herbal formulas that improve cognition in dementia rated the following as the top 10 herbs for improving memory: *Poria cocos*, *Radix et*

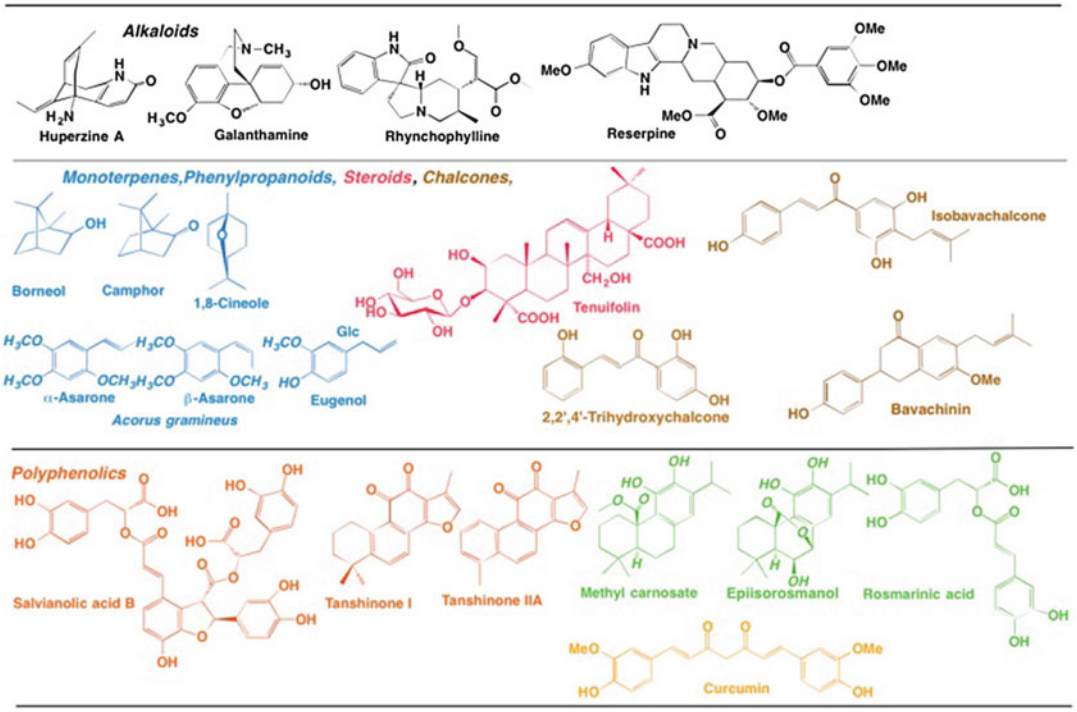


Fig. 5.4 Structures of the major chemical families of active constituents found in Chinese herbs having anti-dementia and β-amyloid anti-aggregation activities

rhizome ginseng, Radix polygalae, Radix et rhizome glycyrrhizae, Radix Angelica sinensis, Rhizoma acori tatarinowii, Semen ziziphi spinosae, Radix rehmanniae, Radix ophiopogonis and Rhizoma zingiberis (Lin et al. 2012; Shen and Chen 2013). The anti-Aβ bioactivity and neuroprotective mechanisms of many of these herbs are outlined in Table 5.3. In Schemes 5.4 and 5.5, the focus is on the particular herbs and spices that can effectively protect against amyloid disease. Their Aβ disaggregation properties and inhibition of tau protein hyperphosphorylation are highlighted (Yoshida et al. 2014; Xian et al. 2012; Fujiwara et al. 2006; Frydman-Marom et al. 2011; Kumaraswamy et al. 2013; Airoldi et al. 2013; Zeng et al. 2013).

5.2.7 Chinese Herbal Formulae for Anti-dementia Protection

Baicalin, jasminoidin, and cholic acid structures (Fig. 5.5) are the main active components of Qingkailing (QKL, Scheme 5.6). QKL is one of the most well-known Chinese herbs and is an aqueous preparation containing extracts of 7 herbs (Cheng et al. 2012). Baicalin is a strong antioxidant; jasminoidin elicits a protective effect on neurons under a broad range of stresses and cholic acid strongly promotes the expression of growth factors in the brain. Upon further investigation of the therapeutic effects and molecular mechanisms of a combination of the three components baicalin, jasminoidin and cholic acid (CBJC) in a rat dementia model, it was found that they significantly up-regulated genes in the forebrain related to neurogenesis and antioxidant neuroprotection (Zhang et al. 2013a).

Kai-xin-san (KXS), a Chinese herbal decoction contains *Ginseng Radix rhizoma*, *R. Polygalae radix*, *R. Acori Tatarinowii*, and *Poria*. KXS has been used in China to treat stress-related psychiatric diseases with the symptoms of depression and forgetfulness. A chemically-standardized water extract of KXS applied to astrocytes significantly stimulated the

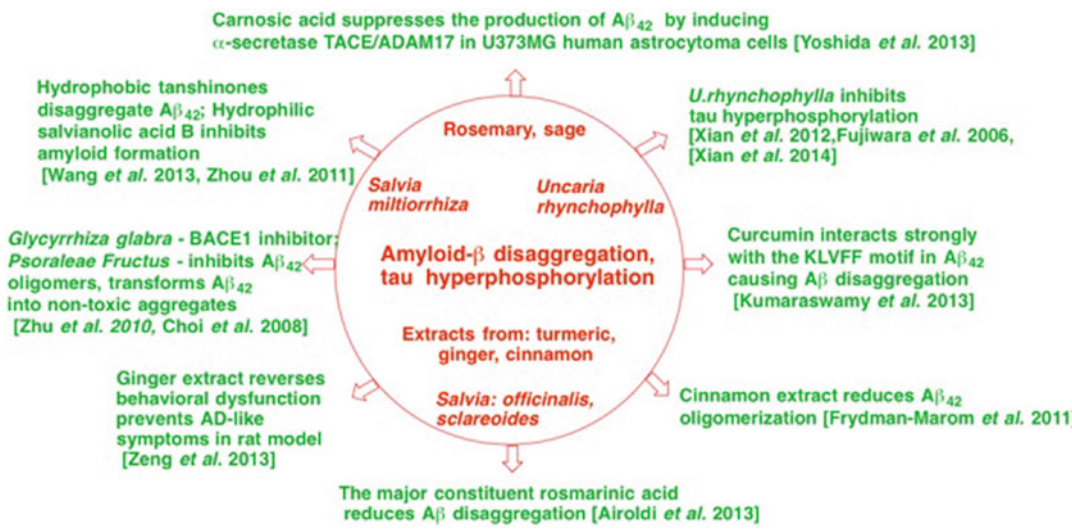
Table 5.3 The top Chinese herbs for improving memory, their major constituents, anti-dementia and neuroprotective actions

Chinese herbs and constituents	Therapeutic and anti-dementia bioactivities
P. cocos (a medicinal mushroom) triterpenes, pachymic acid, dehydropachymic acid.	Antioxidant; water extract enhanced hippocampal long-term potentiation, improved scopolamine-induced spatial memory impairment in rats (Cheng et al. 2013b; Hatip-Al-Khatib et al. 2004; Smriga et al. 1995)
Radix ginseng	Refer to Table 5.2
Radix polygalae (RP) oligosaccharide multi-esters, sucrose esters, triterpene onjisaponins, xanthone and xanthone C-glycosides	Sedative, antipsychotic, cognitive-improving, neuroprotective, with anti-inflammatory therapeutic effects on the central nervous system. Onjisaponin B was able to induce autophagy and accelerate both the removal of mutant huntingtin and A53T α-synuclein, associated with Huntington's and Parkinson's diseases (Ling et al. 2013; Wu et al. 2013a)
Radix Glycyrrhizae (RG) and the active constituent isoliquiritigenin	RG antioxidant activity related to flavonoids and total phenolics (Li et al. 2013b). Prevented Aβ(25–35)-induced neuronal apoptotic death by interfering with the increases of intracellular Ca^{2+} and ROS, and RG potential therapeutic for preventing the progression of AD (Lee et al. 2012)
Radix glycyrrhizae glabra	Administration of 150 and 225 mg/kg improved learning and memory via antioxidant, anti-inflammatory effects in rat model studies. Glycyrrhiza (60–200 μg/mL) contributed to the suppression of Aβ oligomer-induced neuronal damage, DNA fragmentation, and caspase-3 activation (Chakravarthi and Avadhani 2013; Kanno et al. 2013)
R. angelica sinensis (RAS); *Z*-Ligustilide (Lig) (Fig. 5.3) is the major constituent of the lipophilic extract of RAS	Decreased Aβ content and deposition in SAMP8 mice (Huang et al. 2008; Kuang et al. 2006; Hu et al. 2012b)
Semen ziziphi spinosae Jujuboside A (JuA) a major hypnotic-sedative	JuA has shown notable neuroprotective activities via anti-oxidative and anti-inflammatory effects in dementia animals and has potential utilization for the therapeutic treatment of AD (Liu et al. 2014)
Radix Rhemanniae Catalpol, iridoid glycoside	Catalpol reversed brain damage and memory deficits in mice; antioxidant, anti-inflammatory, neurogenetic, antiapoptotic, neuroprotective activities (Liang et al. 2009)
Rhizoma zingiberis ginger root extract (GRE)	GRE reverses behavioral dysfunction and prevents AD-like symptoms in rat model. Ginger has been shown to possess free radical scavenging, antioxidant inhibition of lipid peroxidation, dementia and multiple other therapeutic applications (Zeng et al. 2013; Haniadka et al. 2013)

expression and secretion of neurotrophic factors, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF), in a dose-dependent manner: the stimulation was both in mRNA and protein expression (Zhu et al. 2013; Man et al. 2012). *Rhizoma Acori Tatarinowii* (grassleaf or sweet-flag rhizome), the rhizome of *Acorus tatarinowii Schott,* is used in TCM as an anti-convulsant; it can prevent convulsions as well as convulsion-related GABAergic neuron damage in the brain (Liao et al. 2005).

From the analysis of 1,232 traditional Chinese medicine formulae for anti-dementia (Kong et al. 2009) it was suggested that the most commonly used herbal formulation (Fig. 5.5) was *Rhizoma Chuanxiong, Radix Salviae Miltiorrhizae, Radix Polygalae Tenuifoliae and Rhizoma Acori Tatarinowii.* Their major chemical constituents and anti-AD activities are summarized in Table 5.4.

Yukukansan (Yigan San) is a classical TCM formula used for dementia (Iwasaki et al. 2005b) composed of seven herbs, *Angelica acutiloba*, *Atractylodes lancea*, *Bupleurum falcatum*, *Poria cocos*, *Cnidium officinale*, *Uncaria rhynchophylla* and *Glycyrrhiza uralensis*, in a ratio of 3:4:2:4:3:3:1.5. Clinical randomized controlled trials (RCTs) revealed that Yigan San improved behavioral and psychological symptoms of dementia that include aggression,



Scheme 5.4 Anti β-amyloid effects of food spices and herbs

Scheme 5.5 Chinese herbs most commonly used against dementia and AD

agitation, screaming, wandering, hallucinations and delusions. Yigan San reduces cholinesterase inhibitor-resistant visual hallucinations in dementia patients (Iwasaki et al. 2005a). Yigan San improved psychiatric symptoms and sleep structure in dementia patients (Shinno et al. 2008). The mechanisms of action are related to regulating multiple signal pathways, such as the glutamatergic neurotransmitter system, the serotonin receptor and excitotoxicity (Ho et al. 2011).

A key challenge in validating and translating fundamental science of herbal medicines into better anti-dementia outcomes is to evaluate and scrutinize clinical trial outcomes using scientific research methodologies. Some animal and clinical research performed on herbs leading to improved cognitive health providing options for dementia management and prevention is presented in Table 5.5.

5.3 Summary and Future Outlook

The individual-based interventionist approach against dementia and AD for extending healthy life — better diet and regular exercise — is effective, however it needs much greater promotion, acceptance and adoption early on in life. Alkaloids, monoterpenes, diterpenes, triterpenes, flavonoids, and polyphenolic compounds represent the most prevalent classes of herbal constituents with anti-AD bioactivity. It is unclear to what extent many of these bioactive phytochemicals utilized in single or herbal formulae doses can reach the brain in sufficient concentrations, and in a biologically active form, to exert their beneficial neuroprotective effects. The majority of herbs are consumed as aqueous extracts

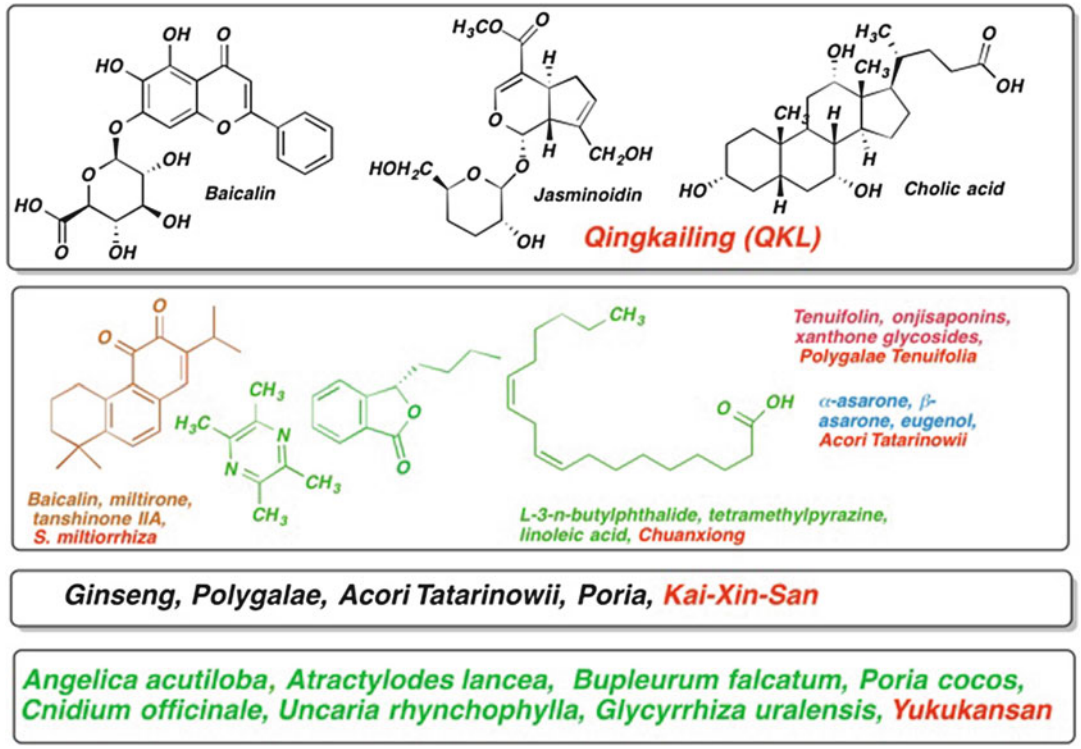
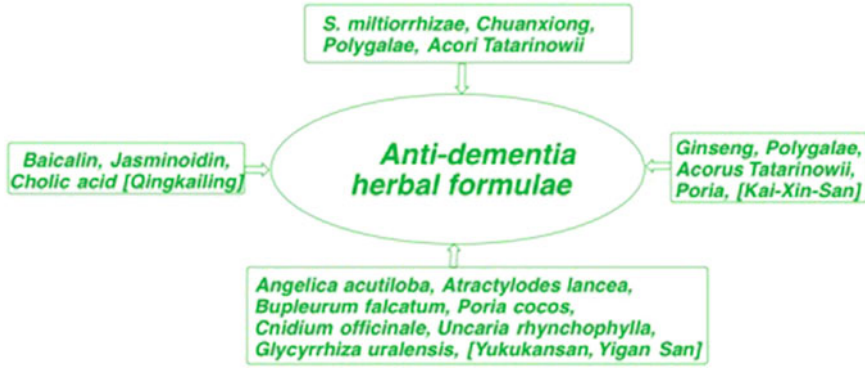


Fig. 5.5 Neuroprotective constituents of Chinese herbal formulae against dementia



Scheme 5.6 Herbal combinations and formulations used for dementia treatment

so their formulation has to provide increased bioavailability and BBB permeability (Hugel and Jackson 2014). An overview of the metabolism and strategies for enhancing polyphenol bioavailability (Lewandowska et al. 2013) include encapsulation of phospholipid-polyphenol complexes; formation of inclusion complexes with cyclodextrins or dendrimers; use of bioactive analogues; derivatisation (e.g., amidation); use of adjuvants (e.g. piperine) as absorption enhancers; and transdermal delivery systems.

It is imperative that herbs and herbal constituents are consumed regularly and in sufficient quantities in the diet. Indeed, for *in vivo* and clinical studies, producing active compounds and extracts in large quantities is an important

Table 5.4 Neuroprotective effects of the four herb TCM formulae commonly used for dementia treatment

The constituents of a four herb anti-dementia TCM formula	Anti-AD activities
Rhizoma Chuanxiong Tetramethylpyrazine ligustrazine	Improved hippocampal cholinergic system function, antioxidant, enhanced learning and memory in AD mice model (Zhao et al. 2008; Shi et al. 2012)
L-3-*n*-butylphthalide (86,89) 9-*cis*, 12-*cis*-linoleic acid (CLA)	*L*-3-n-butylphthalide has been shown to reduce β-amylase-induced neuronal apoptosis, improve cognitive function, blood flow in AD animal models
	CLA as a μ-calpain-specific inhibitor. CLA showed neuroprotective effects against neurotoxins such as H_2O_2 and Aβ(1–42) in SH-SY5Y cells; inhibited Aβ oligomerization and fibrillation. CLA decreased the levels of pro-apoptotic proteins (Lee et al. 2013)
Radix Salviae Miltiorrhizae Baicalin, polyphenolic acids, tanshinones	Antioxidants, anti-inflammatory, neuroprotection; inhibition of Aβ aggregation, oligomerization, and fibril formation (Wang et al. 2013; Zhou et al. 2011; Mei et al. 2009)
Radix Polygalae Tenuifoliae 3,6′-di-O-sinapoyl-sucrose (DISS) tenuifolin, onjisaponins, xanthone glycosides	DISS exerts neuroprotective effects against glutamate toxicity. Reinforces cognitive performance in aged and dysmnesia mice, elevating levels of dopamine, norepinephrine. Onjisaponins indicated cytoprotective activity in PC12 cells, exposed to serum deficiency or glutamate; improved memory in rats by enhancing cholinergic function, inhibiting Aβ secretion (Hu et al. 2009, 2012a; Lin et al. 2012)
Rhizoma Acori Tatarinowii Eugenol, α-asarone, β-asarone	Eugenol derived from *Rhizoma Acori Tatarinowii* increased BDNF mRNA expression level in hippocampus of mice. Modified Wen-Dan-Tang decoction containing *Acori Tatarinowii* attenuated the neurotoxicity of Aβ(25–35) and rescued neurons via suppressing apoptotic process (Liu et al. 2009)

Table 5.5 Clinical trials with herbs to counteract dementia and AD

Natural product	Animal studies; bioactivity mechanisms	Clinical trials
EGCG		300 mg/day of EGCG for 12 weeks had no adverse effect on liver function; did not enhance energy-restricted diet-induced adiposity reductions; did not improve weight-loss-induced changes in cardio-metabolic risk factors in obese Caucasian women (Mielgo-Ayuso et al. 2014)
Huperzine A (alkaloid shown in Fig. 5.3)	APPswe/PS1dE9 transgenic mice reduced Aβ fibrils, oligomers; inhibition of BACE1, regulating APP metabolism (Smriga et al. 1995). EGCG addition to huperzine A, significantly enhanced and prolonged the AChEI effects of huperzine A (Wang et al. 2012; Xiao et al. 2008)	Commonly used in China. USA clinical data (Ha et al. 2011) suggests 0.4 mg doses are required. Further non-Chinese clinical trials are necessary before the implementation of huperzine A for dementia and AD treatment (Yue et al. 2012)
		Systematic review and meta-analysis of 20 RCTs of Huperzine A for AD. Huperzine A appears to have beneficial effects on improvement of cognitive function, daily living activity, and global clinical assessment in participants with AD. The quality of some of the trials was an issue (Yang et al. 2013)

(continued)

Table 5.5 (continued)

Natural product	Animal studies; bioactivity mechanisms	Clinical trials
Curcumin	Curcumin *in vitro* inhibits: Aβ aggregation, Aβ-induced inflammation; the activity of β-secretase; AChE. In *in vivo* studies: oral curcumin inhibition of Aβ deposition, oligomerization, tau phosphorylation in AD animal models. Improvement in behavioral impairment in animal models (Hamaguchi et al. 2010)	Safe to use at dosage of 8 g/day for 3 months
		RCT study on 34 AD patients found no cognitive improvement, increase in anti-oxidant activity and vitamin E levels (Baum et al. 2008)
		Two CTs performed in China and USA have reported no significant differences in changes in cognitive function between placebo and curcumin groups (Gupta et al. 2013)
Korean red ginseng (KRG)		Used for adjuvant treatment for cognitive impairment in AD patients. High-dose KRG (9 g/day, $n = 15$) patients showed significant improvement on the AD Assessment and Clinical Dementia Rating Scale after 12 weeks of KRG therapy (Heo et al. 2008)
Rosemary (*Rosmarinus officinalis L.*; carnosic and rosmarinic acids)		Cognition improving effects of dried rosemary leaf powder on 28 adults (mean age 75 years). Only the lowest dose (750 mg) of rosemary had a statistically significant beneficial effect compared with placebo. Requires further work on effects of low doses over the longer term (Pengelly et al. 2012)

challenge for the utilization of natural products as therapeutic agents. Generally speaking, herbal products offer a wide range of brain-targets, nutritional benefits, safe dosage, long-term applications and efficacious treatment of AD pathology. The focus on engagement of sustainable optimal biochemical performance through diet and factors influencing it, including lifestyle choices, are key to a better mental health.

References

Airoldi C, Sironi E, Dias C, Marcelo F, Martins A, Rauter AP, Nicotra F, Jimenez-Barbero J (2013) Natural compounds against Alzheimer's disease: molecular recognition of Abeta1-42 peptide by Salvia sclareoides extract and its major component, rosmarinic acid, as investigated by NMR. Chem Asian J 8(3):596–602. doi:10.1002/asia.201201063

Bae EA, Hyun YJ, Choo MK, Oh JK, Ryu JH, Kim DH (2004) Protective effect of fermented red ginseng on a transient focal ischemic rats. Arch Pharm Res 27(11):1136–1140

Bao XQ, Kong XC, Qian C, Zhang D (2012) FLZ protects dopaminergic neuron through activating protein kinase B/mammalian target of rapamycin pathway and inhibiting RTP801 expression in Parkinson's disease models. Neuroscience 202:396–404. doi:10.1016/j.neuroscience.2011.11.036

Bao XQ, Li N, Wang T, Kong XC, Tai WJ, Sun H, Zhang D (2013) FLZ alleviates the memory deficits in transgenic mouse model of Alzheimer's disease via decreasing beta-amyloid production and tau hyperphosphorylation. PLoS One 8(11), e78033. doi:10.1371/journal.pone.0078033

Baum L, Lam CW, Cheung SK, Kwok T, Lui V, Tsoh J, Lam L, Leung V, Hui E, Ng C, Woo J, Chiu HF, Goggins WB, Zee BC, Cheng KF, Fong CY, Wong A, Mok H, Chow MS, Ho PC, Ip SP, Ho CS, Yu XW, Lai CY, Chan MH, Szeto S, Chan IH, Mok V (2008) Six-month randomized, placebo-controlled, double-blind, pilot clinical trial of curcumin in patients with Alzheimer disease. J Clin Psychopharmacol 28(1):110–113. doi:10.1097/jcp.0b013e318160862c

Chakravarthi KK, Avadhani R (2013) Beneficial effect of aqueous root extract of Glycyrrhiza glabra on learning and memory using different behavioral models: an experimental study. J Nat Sci Biol Med 4(2):420–425. doi:10.4103/0976-9668.117025

Chen F, Eckman EA, Eckman CB (2006) Reductions in levels of the Alzheimer's amyloid beta peptide after oral administration of ginsenosides. FASEB J 20(8):1269–1271. doi:10.1096/fj.05-5530fje

Chen LM, Lin ZY, Zhu YG, Lin N, Zhang J, Pan XD, Chen XC (2012) Ginsenoside Rg1 attenuates beta-amyloid generation via suppressing PPARgamma-regulated BACE1 activity in N2a-APP695 cells. Eur J Pharmacol 675(1–3):15–21. doi:10.1016/j.ejphar.2011.11.039

Chen X, Yang Y, Zhang Y (2013) Isobavachalcone and bavachinin from Psoraleae Fructus modulate Abeta42 aggregation process through different mechanisms

in vitro. FEBS Lett 587(18):2930–2935. doi:10.1016/j.febslet.2013.07.037

Cheng F, Wang X, Lu Y, Zhong X, Zhao Y, Wang Q (2012) Chinese medicine injection qingkailing for treatment of acute ischemia stroke: a systematic review of randomized controlled trials. Evid Based Complement Alternat Med 2012:213172. doi:10.1155/2012/213172

Cheng B, Gong H, Li X, Sun Y, Chen H, Zhang X, Wu Q, Zheng L, Huang K (2013a) Salvianolic acid B inhibits the amyloid formation of human islet amyloid polypeptide and protects pancreatic beta-cells against cytotoxicity. Proteins 81(4):613–621. doi:10.1002/prot.24216

Cheng S, Eliaz I, Lin J, Thyagarajan-Sahu A, Sliva D (2013b) Triterpenes from Poria cocos suppress growth and invasiveness of pancreatic cancer cells through the downregulation of MMP-7. Int J Oncol 42(6):1869–1874. doi:10.3892/ijo.2013.1902

Choi YH, Yon GH, Hong KS, Yoo DS, Choi CW, Park WK, Kong JY, Kim YS, Ryu SY (2008) In vitro BACE-1 inhibitory phenolic components from the seeds of Psoralea corylifolia. Planta Med 74(11):1405–1408. doi:10.1055/s-2008-1081301

Chu S, Gu J, Feng L, Liu J, Zhang M, Jia X, Liu M, Yao D (2014) Ginsenoside Rg5 improves cognitive dysfunction and beta-amyloid deposition in STZ-induced memory impaired rats via attenuating neuroinflammatory responses. Int Immunopharmacol 19(2):317–326. doi:10.1016/j.intimp.2014.01.018

Esatbeyoglu T, Huebbe P, Ernst IM, Chin D, Wagner AE, Rimbach G (2012) Curcumin–from molecule to biological function. Angew Chem 51(22):5308–5332. doi:10.1002/anie.201107724

Fang F, Liu GT (2008) Novel squamosamide derivative (compound FLZ) attenuates Abeta25-35-induced toxicity in SH-SY5Y cells. Acta Pharmacol Sin 29(2):152–160. doi:10.1111/j.1745-7254.2008.00714.x

Fang F, Wang QL, Liu GT (2012) FLZ, synthetic squamosamide cyclic derivative, attenuates memory deficit and pathological changes in mice with experimentally induced aging. Naunyn Schmiedebergs Arch Pharmacol 385(6):579–585. doi:10.1007/s00210-012-0745-z

Feng Z, Lu Y, Wu X, Zhao P, Li J, Peng B, Qian Z, Zhu L (2012) Ligustilide alleviates brain damage and improves cognitive function in rats of chronic cerebral hypoperfusion. J Ethnopharmacol 144(2):313–321. doi:10.1016/j.jep.2012.09.014

Frydman-Marom A, Levin A, Farfara D, Benromano T, Scherzer-Attali R, Peled S, Vassar R, Segal D, Gazit E, Frenkel D, Ovadia M (2011) Orally administrated cinnamon extract reduces beta-amyloid oligomerization and corrects cognitive impairment in Alzheimer's disease animal models. PLoS One 6(1), e16564. doi:10.1371/journal.pone.0016564

Fujiwara H, Iwasaki K, Furukawa K, Seki T, He M, Maruyama M, Tomita N, Kudo Y, Higuchi M, Saido TC, Maeda S, Takashima A, Hara M, Ohizumi Y, Arai H (2006) Uncaria rhynchophylla, a Chinese medicinal herb, has potent antiaggregation effects on Alzheimer's beta-amyloid proteins. J Neurosci Res 84(2):427–433. doi:10.1002/jnr.20891

Giunta B, Hou H, Zhu Y, Salemi J, Ruscin A, Shytle RD, Tan J (2010) Fish oil enhances anti-amyloidogenic properties of green tea EGCG in Tg2576 mice. Neurosci Lett 471(3):134–138. doi:10.1016/j.neulet.2010.01.026

Gomez-Pinilla F, Tyagi E (2013) Diet and cognition: interplay between cell metabolism and neuronal plasticity. Curr Opin Clin Nutr Metab Care 16(6):726–733. doi:10.1097/MCO.0b013e328365aae3

Green RJ, Murphy AS, Schulz B, Watkins BA, Ferruzzi MG (2007) Common tea formulations modulate in vitro digestive recovery of green tea catechins. Mol Nutr Food Res 51(9):1152–1162. doi:10.1002/mnfr.200700086

Gupta SC, Patchva S, Aggarwal BB (2013) Therapeutic roles of curcumin: lessons learned from clinical trials. AAPS J 15(1):195–218. doi:10.1208/s12248-012-9432-8

Ha GT, Wong RK, Zhang Y (2011) Huperzine a as potential treatment of Alzheimer's disease: an assessment on chemistry, pharmacology, and clinical studies. Chem Biodivers 8(7):1189–1204. doi:10.1002/cbdv.201000269

Hamaguchi T, Ono K, Yamada M (2010) REVIEW: curcumin and Alzheimer's disease. CNS Neurosci Ther 16(5):285–297. doi:10.1111/j.1755-5949.2010.00147.x

Haniadka R, Saldanha E, Sunita V, Palatty PL, Fayad R, Baliga MS (2013) A review of the gastroprotective effects of ginger (Zingiber officinale Roscoe). Food Function 4(6):845–855. doi:10.1039/c3fo30337c

Hatip-Al-Khatib I, Egashira N, Mishima K, Iwasaki K, Iwasaki K, Kurauchi K, Inui K, Ikeda T, Fujiwara M (2004) Determination of the effectiveness of components of the herbal medicine Toki-Shakuyaku-San and fractions of Angelica acutiloba in improving the scopolamine-induced impairment of rat's spatial cognition in eight-armed radial maze test. J Pharmacol Sci 96(1):33–41

Heo JH, Lee ST, Chu K, Oh MJ, Park HJ, Shim JY, Kim M (2008) An open-label trial of Korean red ginseng as an adjuvant treatment for cognitive impairment in patients with Alzheimer's disease. Eur J Neurol 15(8):865–868. doi:10.1111/j.1468-1331.2008.02157.x

Ho YS, So KF, Chang RC (2011) Drug discovery from Chinese medicine against neurodegeneration in Alzheimer's and vascular dementia. Chin Med 6:15. doi:10.1186/1749-8546-6-15

Hu Y, Liao HB, Liu P, Guo DH, Rahman K (2009) A bioactive compound from Polygala tenuifolia regulates efficiency of chronic stress on hypothalamic-pituitary-adrenal axis. Pharmazie 64(9):605–608

Hu Y, Li J, Liu P, Chen X, Guo DH, Li QS, Rahman K (2012a) Protection of SH-SY5Y neuronal cells from glutamate-induced apoptosis by 3,6′-disinapoyl sucrose, a bioactive compound isolated from Radix

Polygala. J Biomed Biotechnol 2012:1–5. doi:10.1155/2012/728342

Hu ZY, Liu G, Cheng XR, Huang Y, Yang S, Qiao SY, Sun L, Zhou WX, Zhang YX (2012b) JD-30, an active fraction extracted from Danggui-Shaoyao-San, decreases beta-amyloid content and deposition, improves LTP reduction and prevents spatial cognition impairment in SAMP8 mice. Exp Gerontol 47(1):14–22. doi:10.1016/j.exger.2011.09.009

Hu B, Ting Y, Zeng X, Huang Q (2013) Bioactive peptides/chitosan nanoparticles enhance cellular antioxidant activity of (−)-epigallocatechin-3-gallate. J Agric Food Chem 61(4):875–881. doi:10.1021/jf304821k

Huai Y, Dong Y, Xu J, Meng N, Song C, Li W, Lv P (2013) L-3-n-butylphthalide protects against vascular dementia via activation of the Akt kinase pathway. Neural Regen Res 8(19):1733–1742. doi:10.3969/j.issn.1673-5374.2013.19.001

Huang SH, Lin CM, Chiang BH (2008) Protective effects of Angelica sinensis extract on amyloid beta-peptide-induced neurotoxicity. Phytomedicine 15(9):710–721. doi:10.1016/j.phymed.2008.02.022

Huang T, Fang F, Chen L, Zhu Y, Zhang J, Chen X, Yan SS (2012) Ginsenoside Rg1 attenuates oligomeric Abeta(1–42)-induced mitochondrial dysfunction. Curr Alzheimer Res 9(3):388–395

Huang HC, Tang D, Xu K, Jiang ZF (2014a) Curcumin attenuates amyloid-beta-induced tau hyperphosphorylation in human neuroblastoma SH-SY5Y cells involving PTEN/Akt/GSK-3beta signaling pathway. J Recept Signal Transduct Res 34(1):26–37. doi:10.3109/10799893.2013.848891

Huang J, Wu D, Wang J, Li F, Lu L, Gao Y, Zhong Z (2014b) Effects of Panax notoginseng saponin on alpha, beta, and gamma secretase involved in Abeta deposition in SAMP8 mice. Neuroreport 25(2):89–93. doi:10.1097/WNR.0000000000000048

Hugel HM, Jackson N (2012) Redox chemistry of green tea polyphenols: therapeutic benefits in neurodegenerative diseases. Mini Rev Med Chem 12(5):380–387

Hugel HM, Jackson N (2014) Danshen diversity defeating dementia. Bioorg Med Chem Lett 24(3):708–716. doi:10.1016/j.bmcl.2013.12.042

Hugel HM, Jackson N, May BH, Xue CC (2012) Chinese herbs for dementia diseases. Mini Rev Med Chem 12(5):371–379

Hyung SJ, DeToma AS, Brender JR, Lee S, Vivekanandan S, Kochi A, Choi JS, Ramamoorthy A, Ruotolo BT, Lim MH (2013) Insights into antiamyloidogenic properties of the green tea extract (−)-epigallocatechin-3-gallate toward metal-associated amyloid-beta species. Proc Natl Acad Sci U S A 110(10):3743–3748. doi:10.1073/pnas.1220326110

International WHOaAsD (2012) Dementia: a public health priority. WHO Regional Office for Europe, Copenhagen

Iwasaki K, Maruyama M, Tomita N, Furukawa K, Nemoto M, Fujiwara H, Seki T, Fujii M, Kodama M, Arai H (2005a) Effects of the traditional Chinese herbal medicine Yi-Gan San for cholinesterase inhibitor-resistant visual hallucinations and neuropsychiatric symptoms in patients with dementia with Lewy bodies. J Clin Psychiatry 66(12):1612–1613

Iwasaki K, Satoh-Nakagawa T, Maruyama M, Monma Y, Nemoto M, Tomita N, Tanji H, Fujiwara H, Seki T, Fujii M, Arai H, Sasaki H (2005b) A randomized, observer-blind, controlled trial of the traditional Chinese medicine Yi-Gan San for improvement of behavioral and psychological symptoms and activities of daily living in dementia patients. J Clin Psychiatry 66(2):248–252

Jayasena T, Poljak A, Smythe G, Braidy N, Munch G, Sachdev P (2013) The role of polyphenols in the modulation of sirtuins and other pathways involved in Alzheimer's disease. Ageing Res Rev 12(4):867–883. doi:10.1016/j.arr.2013.06.003

Kakkar V, Mishra AK, Chuttani K, Kaur IP (2013) Proof of concept studies to confirm the delivery of curcumin loaded solid lipid nanoparticles (C-SLNs) to brain. Int J Pharm 448(2):354–359. doi:10.1016/j.ijpharm.2013.03.046

Kang RX, Zhang JJ (2012) A natural squamosamide derivative FLZ inhibits homocysteine-induced rat brain microvascular endothelial cells dysfunction. Biochem Biophys Res Commun 417(4):1176–1181. doi:10.1016/j.bbrc.2011.12.094

Kang MS, Baek SH, Chun YS, Moore AZ, Landman N, Berman D, Yang HO, Morishima-Kawashima M, Osawa S, Funamoto S, Ihara Y, Di Paolo G, Park JH, Chung S, Kim TW (2013) Modulation of lipid kinase PI4KIIalpha activity and lipid raft association of presenilin 1 underlies gamma-secretase inhibition by ginsenoside (20S)-Rg3. J Biol Chem 288(29):20868–20882. doi:10.1074/jbc.M112.445734

Kanno H, Kawakami Z, Iizuka S, Tabuchi M, Mizoguchi K, Ikarashi Y, Kase Y (2013) Glycyrrhiza and Uncaria Hook contribute to protective effect of traditional Japanese medicine yokukansan against amyloid beta oligomer-induced neuronal death. J Ethnopharmacol 149(1):360–370. doi:10.1016/j.jep.2013.06.052

Karpagam V, Sathishkumar N, Sathiyamoorthy S, Rasappan P, Shila S, Kim YJ, Yang DC (2013) Identification of BACE1 inhibitors from Panax ginseng saponins-An Insilco approach. Comput Biol Med 43(8):1037–1044. doi:10.1016/j.compbiomed.2013.05.009

Kim DS, Kim JY, Han YS (2007) Alzheimer's disease drug discovery from herbs: neuroprotectivity from beta-amyloid (1–42) insult. J Altern Complement Med 13(3):333–340. doi:10.1089/acm.2006.6107

Kim HJ, Kim P, Shin CY (2013a) A comprehensive review of the therapeutic and pharmacological effects of ginseng and ginsenosides in central nervous system. J Ginseng Res 37(1):8–29. doi:10.5142/jgr.2013.37.8

Kim J, Kim SH, Lee DS, Lee DJ, Kim SH, Chung S, Yang HO (2013b) Effects of fermented ginseng on memory impairment and beta-amyloid reduction in Alzheimer's disease experimental models. J Ginseng Res 37(1):100–107. doi:10.5142/jgr.2013.37.100

Kong DX, Li XJ, Zhang HY (2009) Where is the hope for drug discovery? Let history tell the future. Drug Discov Today 14(3–4):115–119. doi:10.1016/j.drudis.2008.07.002

Kong XC, Zhang D, Qian C, Liu GT, Bao XQ (2011) FLZ, a novel HSP27 and HSP70 inducer, protects SH-SY5Y cells from apoptosis caused by MPP(+). Brain Res 1383:99–107. doi:10.1016/j.brainres.2011.01.093

Kuang X, Yao Y, Du JR, Liu YX, Wang CY, Qian ZM (2006) Neuroprotective role of Z-ligustilide against forebrain ischemic injury in ICR mice. Brain Res 1102(1):145–153. doi:10.1016/j.brainres.2006.04.110

Kumaraswamy P, Sethuraman S, Krishnan UM (2013) Mechanistic insights of curcumin interactions with the core-recognition motif of beta-amyloid peptide. J Agric Food Chem 61(13):3278–3285. doi:10.1021/jf4000709

Lambert JD, Sang S, Hong J, Kwon SJ, Lee MJ, Ho CT, Yang CS (2006) Peracetylation as a means of enhancing in vitro bioactivity and bioavailability of epigallocatechin-3-gallate. Drug Metab Dispos 34(12):2111–2116. doi:10.1124/dmd.106.011460

Lee SH, Jung BH, Choi SY, Kim SY, Lee EH, Chung BC (2006a) Influence of ginsenoside Rb1 on brain neurosteroid during acute immobilization stress. Arch Pharm Res 29(7):566–569

Lee SH, Jung BH, Kim SY, Lee EH, Chung BC (2006b) The antistress effect of ginseng total saponin and ginsenoside Rg3 and Rb1 evaluated by brain polyamine level under immobilization stress. Pharmacol Res 54(1):46–49. doi:10.1016/j.phrs.2006.02.001

Lee YK, Yuk DY, Lee JW, Lee SY, Ha TY, Oh KW, Yun YP, Hong JT (2009) (−)-Epigallocatechin-3-gallate prevents lipopolysaccharide-induced elevation of beta-amyloid generation and memory deficiency. Brain Res 1250:164–174. doi:10.1016/j.brainres.2008.10.012

Lee YJ, Choi IS, Park MH, Lee YM, Song JK, Kim YH, Kim KH, Hwang DY, Jeong JH, Yun YP, Oh KW, Jung JK, Han SB, Hong JT (2011a) 4-O-Methylhonokiol attenuates memory impairment in presenilin 2 mutant mice through reduction of oxidative damage and inactivation of astrocytes and the ERK pathway. Free Radic Biol Med 50(1):66–77. doi:10.1016/j.freeradbiomed.2010.10.698

Lee YJ, Lee YM, Lee CK, Jung JK, Han SB, Hong JT (2011b) Therapeutic applications of compounds in the Magnolia family. Pharmacol Ther 130(2):157–176. doi:10.1016/j.pharmthera.2011.01.010

Lee YJ, Choi DY, Han SB, Kim YH, Kim KH, Hwang BY, Kang JK, Lee BJ, Oh KW, Hong JT (2012) Inhibitory effect of ethanol extract of Magnolia officinalis on memory impairment and amyloidogenesis in a transgenic mouse model of Alzheimer's disease via regulating beta-secretase activity. Phytother Res 26(12):1884–1892. doi:10.1002/ptr.4643

Lee E, Eom JE, Kim HL, Baek KH, Jun KY, Kim HJ, Lee M, Mook-Jung I, Kwon Y (2013) Effect of conjugated linoleic acid, mu-calpain inhibitor, on pathogenesis of Alzheimer's disease. Biochim Biophys Acta 1831(4):709–718. doi:10.1016/j.bbalip.2012.12.003

Lewandowska U, Szewczyk K, Hrabec E, Janecka A, Gorlach S (2013) Overview of metabolism and bioavailability enhancement of polyphenols. J Agric Food Chem 61(50):12183–12199. doi:10.1021/jf404439b

Li N, Liu GT (2010) The novel squamosamide derivative FLZ enhances BDNF/TrkB/CREB signaling and inhibits neuronal apoptosis in APP/PS1 mice. Acta Pharmacol Sin 31(3):265–272. doi:10.1038/aps.2010.3

Li B, Du W, Jin J, Du Q (2012) Preservation of (−)-epigallocatechin-3-gallate antioxidant properties loaded in heat treated beta-lactoglobulin nanoparticles. J Agric Food Chem 60(13):3477–3484. doi:10.1021/jf300307t

Li L, Liu Z, Liu J, Tai X, Hu X, Liu X, Wu Z, Zhang G, Shi M, Zhao G (2013a) Ginsenoside Rd attenuates beta-amyloid-induced tau phosphorylation by altering the functional balance of glycogen synthase kinase 3beta and protein phosphatase 2A. Neurobiol Dis 54:320–328. doi:10.1016/j.nbd.2013.01.002

Li X, Chen W, Chen D (2013b) Protective effect against hydroxyl-induced DNA damage and antioxidant activity of radix glycyrrhizae (liquorice root). Adva Pharm Bull 3(1):167–173. doi:10.5681/apb.2013.028

Liang JH, Du J, Xu LD, Jiang T, Hao S, Bi J, Jiang B (2009) Catalpol protects primary cultured cortical neurons induced by Abeta(1–42) through a mitochondrial-dependent caspase pathway. Neurochem Int 55(8):741–746. doi:10.1016/j.neuint.2009.07.004

Liao WP, Chen L, Yi YH, Sun WW, Gao MM, Su T, Yang SQ (2005) Study of antiepileptic effect of extracts from Acorus tatarinowii Schott. Epilepsia 46(Suppl 1):21–24. doi:10.1111/j.0013-9580.2005.461007.x

Lin Z, Gu J, Xiu J, Mi T, Dong J, Tiwari JK (2012) Traditional Chinese medicine for senile dementia. Evid Based Complement Alternat Med 2012:692621. doi:10.1155/2012/692621

Ling Y, Li Z, Chen M, Sun Z, Fan M, Huang C (2013) Analysis and detection of the chemical constituents of Radix Polygalae and their metabolites in rats after oral administration by ultra high-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry. J Pharm Biomed Anal 85:1–13. doi:10.1016/j.jpba.2013.06.011

Liu P, Zhao L, Zhang SL, Xiang JZ (2009) Modified Wendan decoction can attenuate neurotoxic action associated with Alzheimer's disease. Evid Based Complement Alternat Med 6(3):325–330. doi:10.1093/ecam/nem103

Liu LF, Durairajan SS, Lu JH, Koo I, Li M (2012) In vitro screening on amyloid precursor protein modulation of plants used in Ayurvedic and traditional Chinese medicine for memory improvement. J Ethnopharmacol 141(2):754–760. doi:10.1016/j.jep.2011.08.065

Liu Z, Zhao X, Liu B, Liu AJ, Li H, Mao X, Wu B, Bi KS, Jia Y (2014) Jujuboside A, a neuroprotective agent from semen Ziziphi Spinosae ameliorates behavioral disorders of the dementia mouse model induced by

Abeta 1–42. Eur J Pharmacol 738:206–213. doi:10.1016/j.ejphar.2014.05.041

Lotito SB, Frei B (2006) Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? Free Radic Biol Med 41(12):1727–1746. doi:10.1016/j.freeradbiomed.2006.04.033

Lv J, Jia H, Jiang Y, Ruan Y, Liu Z, Yue W, Beyreuther K, Tu P, Zhang D (2009) Tenuifolin, an extract derived from tenuigenin, inhibits amyloid-beta secretion in vitro. Acta Physiol 196(4):419–425. doi:10.1111/j.1748-1716.2009.01961.x

Man SC, Chan KW, Lu JH, Durairajan SS, Liu LF, Li M (2012) Systematic review on the efficacy and safety of herbal medicines for vascular dementia. Evid Based Complement Alternat Med 2012:426215. doi:10.1155/2012/426215

Mapstone M, Cheema AK, Fiandaca MS, Zhong X, Mhyre TR, MacArthur LH, Hall WJ, Fisher SG, Peterson DR, Haley JM, Nazar MD, Rich SA, Berlau DJ, Peltz CB, Tan MT, Kawas CH, Federoff HJ (2014) Plasma phospholipids identify antecedent memory impairment in older adults. Nat Med 20(4):415–418. doi:10.1038/nm.3466

May BH, Yang AW, Zhang AL, Owens MD, Bennett L, Head R, Cobiac L, Li CG, Hugel H, Story DF, Xue CC (2009) Chinese herbal medicine for mild cognitive impairment and age associated memory impairment: a review of randomised controlled trials. Biogerontology 10(2):109–123. doi:10.1007/s10522-008-9163-5

May BH, Lu C, Bennett L, Hugel HM, Xue CC (2012) Evaluating the traditional Chinese literature for herbal formulae and individual herbs used for age-related dementia and memory impairment. Biogerontology 13(3):299–312. doi:10.1007/s10522-012-9375-6

Mei Z, Zhang F, Tao L, Zheng W, Cao Y, Wang Z, Tang S, Le K, Chen S, Pi R, Liu P (2009) Cryptotanshinone, a compound from Salvia miltiorrhiza modulates amyloid precursor protein metabolism and attenuates beta-amyloid deposition through upregulating alpha-secretase in vivo and in vitro. Neurosci Lett 452(2):90–95. doi:10.1016/j.neulet.2009.01.013

Mielgo-Ayuso J, Barrenechea L, Alcorta P, Larrarte E, Margareto J, Labayen I (2014) Effects of dietary supplementation with epigallocatechin-3-gallate on weight loss, energy homeostasis, cardiometabolic risk factors and liver function in obese women: randomised, double-blind, placebo-controlled clinical trial. Br J Nutr 111(7):1263–1271. doi:10.1017/S0007114513003784

Mourtas S, Lazar AN, Markoutsa E, Duyckaerts C, Antimisiaris SG (2014) Multifunctional nanoliposomes with curcumin-lipid derivative and brain targeting functionality with potential applications for Alzheimer disease. Eur J Med Chem 80:175–183. doi:10.1016/j.ejmech.2014.04.050

Nastase AF, Boyd DB (2012) Simple structure-based approach for predicting the activity of inhibitors of beta-secretase (BACE1) associated with Alzheimer's disease. J Chem Inf Model 52(12):3302–3307. doi:10.1021/ci300331d

Palhano FL, Lee J, Grimster NP, Kelly JW (2013) Toward the molecular mechanism(s) by which EGCG treatment remodels mature amyloid fibrils. J Am Chem Soc 135(20):7503–7510. doi:10.1021/ja3115696

Pang HY, Liu G, Liu GT (2009) Compound FLZ inhibits lipopolysaccharide-induced inflammatory effects via down-regulation of the TAK-IKK and TAK-JNK/p38MAPK pathways in RAW264.7 macrophages. Acta Pharmacol Sin 30(2):209–218. doi:10.1038/aps.2008.29

Peng Y, Xing C, Lemere CA, Chen G, Wang L, Feng Y, Wang X (2008) l-3-n-Butylphthalide ameliorates beta-amyloid-induced neuronal toxicity in cultured neuronal cells. Neurosci Lett 434(2):224–229. doi:10.1016/j.neulet.2008.01.080

Peng Y, Hu Y, Xu S, Li P, Li J, Lu L, Yang H, Feng N, Wang L, Wang X (2012) L-3-n-butylphthalide reduces tau phosphorylation and improves cognitive deficits in AbetaPP/PS1-Alzheimer's transgenic mice. J Alzheimers Dis 29(2):379–391. doi:10.3233/JAD-2011-111577

Peng Y, Hu Y, Xu S, Rong X, Li J, Li P, Wang L, Yang J, Wang X (2014) Potassium 2-(1-hydroxypentyl)-benzoate improves memory deficits and attenuates amyloid and tau pathologies in a mouse model of Alzheimer's disease. J Pharmacol Exp Ther 350(2):361–374. doi:10.1124/jpet.114.213140

Pengelly A, Snow J, Mills SY, Scholey A, Wesnes K, Butler LR (2012) Short-term study on the effects of rosemary on cognitive function in an elderly population. J Med Food 15(1):10–17. doi:10.1089/jmf.2011.0005

Qin Y, Pan X, Tang TT, Zhou L, Gong XG (2011) Anti-proliferative effects of the novel squamosamide derivative (FLZ) on HepG2 human hepatoma cells by regulating the cell cycle-related proteins are associated with decreased Ca(2+)/ROS levels. Chem Biol Interact 193(3):246–253. doi:10.1016/j.cbi.2011.07.004

Quan Q, Wang J, Li X, Wang Y (2013) Ginsenoside Rg1 decreases Abeta(1–42) level by upregulating PPARgamma and IDE expression in the hippocampus of a rat model of Alzheimer's disease. PLoS One 8(3), e59155. doi:10.1371/journal.pone.0059155

Schaffer S, Halliwell B (2012) Do polyphenols enter the brain and does it matter? Some theoretical and practical considerations. Genes Nutr 7(2):99–109. doi:10.1007/s12263-011-0255-5

Shen J, Chen X (2013) Drug discovery from traditional Chinese medicine for neurogenesis: implications for stroke and neurodegenerative diseases. In: Adams JD, Lien EJ (eds) Traditional Chinese medicine: scientific basis for its use. Royal Society of Chemistry Publishing, Cambridge, pp 204–237

Shi J, Wang Y, Luo G (2012) Ligustrazine phosphate ethosomes for treatment of Alzheimer's disease, in vitro and in animal model studies. AAPS Pharm Sci Tech 13(2):485–492. doi:10.1208/s12249-012-9767-6

Shi C, Na N, Zhu X, Xu J (2013) Estrogenic effect of ginsenoside Rg1 on APP processing in postmenopausal platelets. Platelets 24(1):51–62. doi:10.3109/09537104.2012.654839

Shinno H, Inami Y, Inagaki T, Nakamura Y, Horiguchi J (2008) Effect of Yi-Gan San on psychiatric symptoms and sleep structure at patients with behavioral and psychological symptoms of dementia. Prog Neuropsychopharmacol Biol Psychiatry 32(3):881–885. doi:10.1016/j.pnpbp.2007.12.027

Singh M, Arseneault M, Sanderson T, Murthy V, Ramassamy C (2008) Challenges for research on polyphenols from foods in Alzheimer's disease: bioavailability, metabolism, and cellular and molecular mechanisms. J Agric Food Chem 56(13):4855–4873. doi:10.1021/jf0735073

Smith A, Giunta B, Bickford PC, Fountain M, Tan J, Shytle RD (2010) Nanolipidic particles improve the bioavailability and alpha-secretase inducing ability of epigallocatechin-3-gallate (EGCG) for the treatment of Alzheimer's disease. Int J Pharm 389(1–2):207–212. doi:10.1016/j.ijpharm.2010.01.012

Smriga M, Saito H, Nishiyama N (1995) Hoelen (Poria Cocos Wolf) and ginseng (Panax Ginseng C. A. Meyer), the ingredients of a Chinese prescription DX-9386, individually promote hippocampal long-term potentiation in vivo. Biol Pharm Bull 18(4):518–522

Tai W, Ye X, Bao X, Zhao B, Wang X, Zhang D (2013) Inhibition of Src tyrosine kinase activity by squamosamide derivative FLZ attenuates neuroinflammation in both in vivo and in vitro Parkinson's disease models. Neuropharmacology 75:201–212. doi:10.1016/j.neuropharm.2013.07.020

Tiwari SK, Agarwal S, Seth B, Yadav A, Nair S, Bhatnagar P, Karmakar M, Kumari M, Chauhan LK, Patel DK, Srivastava V, Singh D, Gupta SK, Tripathi A, Chaturvedi RK, Gupta KC (2014) Curcumin-loaded nanoparticles potently induce adult neurogenesis and reverse cognitive deficits in Alzheimer's disease model via canonical Wnt/beta-catenin pathway. ACS Nano 8(1):76–103. doi:10.1021/nn405077y

Ulijaszek SJ, Mann N, Elton S (2012) Evolving human nutrition: implications for public health, vol 64. Cambridge studies in biological and evolutionary anthropology. Cambridge University Press, Cambridge. doi:http://dx.doi.org/10.1017/CBO9781139046794

van Duynhoven J, Vaughan EE, Jacobs DM, Kemperman RA, van Velzen EJ, Gross G, Roger LC, Possemiers S, Smilde AK, Dore J, Westerhuis JA, Van de Wiele T (2011) Metabolic fate of polyphenols in the human superorganism. Proc Natl Acad Sci U S A 108(Suppl 1):4531–4538. doi:10.1073/pnas.1000098107

Villeda SA, Luo J, Mosher KI, Zou B, Britschgi M, Bieri G, Stan TM, Fainberg N, Ding Z, Eggel A, Lucin KM, Czirr E, Park JS, Couillard-Despres S, Aigner L, Li G, Peskind ER, Kaye JA, Quinn JF, Galasko DR, Xie XS, Rando TA, Wyss-Coray T (2011) The ageing systemic milieu negatively regulates neurogenesis and cognitive function. Nature 477(7362):90–94. doi:10.1038/nature10357

Wang SH, Liu FF, Dong XY, Sun Y (2010) Thermodynamic analysis of the molecular interactions between amyloid beta-peptide 42 and (−)-epigallocatechin-3-gallate. J Phys Chem B 114(35):11576–11583. doi:10.1021/jp1001435

Wang Y, Tang XC, Zhang HY (2012) Huperzine A alleviates synaptic deficits and modulates amyloidogenic and nonamyloidogenic pathways in APPswe/PS1dE9 transgenic mice. J Neurosci Res 90(2):508–517. doi:10.1002/jnr.22775

Wang Q, Yu X, Patal K, Hu R, Chuang S, Zhang G, Zheng J (2013) Tanshinones inhibit amyloid aggregation by amyloid-beta peptide, disaggregate amyloid fibrils, and protect cultured cells. ACS Chem Neurosci 4(6):1004–1015. doi:10.1021/cn400051e

Wang P, Su C, Li R, Wang H, Ren Y, Sun H, Yang J, Sun J, Shi J, Tian J, Jiang S (2014) Mechanisms and effects of curcumin on spatial learning and memory improvement in APPswe/PS1dE9 mice. J Neurosci Res 92(2):218–231. doi:10.1002/jnr.23322

Wimo A, Jonsson L, Bond J, Prince M, Winblad B, Alzheimer Disease I (2013) The worldwide economic impact of dementia 2010. Alzheimers Dement 9(1):1–11. doi:10.1016/j.jalz.2012.11.006, e13

Witte AV, Fobker M, Gellner R, Knecht S, Floel A (2009) Caloric restriction improves memory in elderly humans. Proc Natl Acad Sci U S A 106(4):1255–1260. doi:10.1073/pnas.0808587106

Wu AG, Wong VK, Xu SW, Chan WK, Ng CI, Liu L, Law BY (2013a) Onjisaponin B derived from Radix Polygalae enhances autophagy and accelerates the degradation of mutant alpha-synuclein and huntingtin in PC-12 cells. Int J Mol Sci 14(11):22618–22641. doi:10.3390/ijms141122618

Wu S, Sun K, Wang X, Wang D, Wan X, Zhang J (2013b) Protonation of epigallocatechin-3-gallate (EGCG) results in massive aggregation and reduced oral bioavailability of EGCG-dispersed selenium nanoparticles. J Agric Food Chem 61(30):7268–7275. doi:10.1021/jf4000083

Xian YF, Lin ZX, Mao QQ, Hu Z, Zhao M, Che CT, Ip SP (2012) Bioassay-guided isolation of neuroprotective compounds from Uncaria rhynchophylla against beta-amyloid-induced neurotoxicity. Evid Based Complement Alternat Med 2012:802625. doi:10.1155/2012/802625

Xiao J, Chen X, Zhang L, Talbot SG, Li GC, Xu M (2008) Investigation of the mechanism of enhanced effect of EGCG on huperzine A's inhibition of acetylcholinesterase activity in rats by a multispectroscopic method. J Agric Food Chem 56(3):910–915. doi:10.1021/jf073036k

Xin J, Zhang J, Yang Y, Deng M, Xie X (2013) Radix Angelica Sinensis that contains the component Z-ligustilide promotes adult neurogenesis to mediate recovery from cognitive impairment. Curr Neurovasc Res 10(4):304–315

Yang L, Hao J, Zhang J, Xia W, Dong X, Hu X, Kong F, Cui X (2009) Ginsenoside Rg3 promotes beta-amyloid peptide degradation by enhancing gene expression of

neprilysin. J Pharm Pharmacol 61(3):375–380. doi:10.1211/jpp/61.03.0013

Yang G, Wang Y, Tian J, Liu JP (2013) Huperzine A for Alzheimer's disease: a systematic review and meta-analysis of randomized clinical trials. PLoS One 8(9), e74916. doi:10.1371/journal.pone.0074916

Ye X, Tai W, Bao X, Chen X, Zhang D (2014) FLZ inhibited gamma-secretase selectively and decreased Abeta mitochondrial production in APP-SH-SY5Y cells. Naunyn Schmiedebergs Arch Pharmacol 387(1):75–85. doi:10.1007/s00210-013-0918-4

Yoshida H, Meng P, Matsumiya T, Tanji K, Hayakari R, Xing F, Wang L, Tsuruga K, Tanaka H, Mimura J, Kosaka K, Itoh K, Takahashi I, Imaizumi T (2014) Carnosic acid suppresses the production of amyloid-beta 1–42 and 1–43 by inducing an alpha-secretase TACE/ADAM17 in U373MG human astrocytoma cells. Neurosci Res 79:83–93. doi:10.1016/j.neures.2013.11.004

Yue J, Dong BR, Lin X, Yang M, Wu HM, Wu T (2012) Huperzine A for mild cognitive impairment. Cochrane Database Syst Rev (12), CD008827. doi:10.1002/14651858.CD008827.pub2

Zeng GF, Zhang ZY, Lu L, Xiao DQ, Zong SH, He JM (2013) Protective effects of ginger root extract on Alzheimer disease-induced behavioral dysfunction in rats. Rejuvenation Res 16(2):124–133. doi:10.1089/rej.2012.1389

Zhang YJ, Wu L, Zhang QL, Li J, Yin FX, Yuan Y (2011) Pharmacokinetics of phenolic compounds of Danshen extract in rat blood and brain by microdialysis sampling. J Ethnopharmacol 136(1):129–136. doi:10.1016/j.jep.2011.04.023

Zhang J, Li P, Wang Y, Liu J, Zhang Z, Cheng W, Wang Y (2013a) Ameliorative effects of a combination of baicalin, jasminoidin and cholic acid on ibotenic acid-induced dementia model in rats. PLoS One 8(2), e56658. doi:10.1371/journal.pone.0056658

Zhang SQ, Sawmiller D, Li S, Rezai-Zadeh K, Hou H, Zhou S, Shytle D, Giunta B, Fernandez F, Mori T, Tan J (2013b) Octyl gallate markedly promotes anti-amyloidogenic processing of APP through estrogen receptor-mediated ADAM10 activation. PLoS One 8(8), e71913. doi:10.1371/journal.pone.0071913

Zhang X, Tian Y, Li Z, Tian X, Sun H, Liu H, Moore A, Ran C (2013c) Design and synthesis of curcumin analogues for in vivo fluorescence imaging and inhibiting copper-induced cross-linking of amyloid beta species in Alzheimer's disease. J Am Chem Soc 135(44):16397–16409. doi:10.1021/ja405239v

Zhang Z, Du GJ, Wang CZ, Wen XD, Calway T, Li Z, He TC, Du W, Bissonnette M, Musch MW, Chang EB, Yuan CS (2013d) Compound K, a ginsenoside metabolite, inhibits colon cancer growth via multiple pathways including p53-p21 interactions. Int J Mol Sci 14(2):2980–2995. doi:10.3390/ijms14022980

Zhang XY, Qiao H, Shi YB (2014) HPLC method with fluorescence deduction for the determination of ligustilide in rat plasma and its pharmacokinetics. Pharm Biol 52:21–30

Zhao L, Wei MJ, He M, Jin WB, Zhai HS, Yao WF (2008) The effects of tetramethylpyrazine on learning and memory abilities of mice with Alzheimer's disease and its possible mechanism. Chin Pharmacol Bull 24:1088–1092

Zhao W, Xu S, Peng Y, Ji X, Cao D, Li J, Liu B, Shi Q, Wang L, Wang X (2013) Potassium 2-(1-hydroxypentyl)-benzoate improves learning and memory deficits in chronic cerebral hypoperfused rats. Neurosci Lett 541:155–160. doi:10.1016/j.neulet.2013.01.053

Zhou Y, Li W, Xu L, Chen L (2011) In Salvia miltiorrhiza, phenolic acids possess protective properties against amyloid beta-induced cytotoxicity, and tanshinones act as acetylcholinesterase inhibitors. Environ Toxicol Pharmacol 31(3):443–452. doi:10.1016/j.etap.2011.02.006

Zhou L, Hou Y, Yang Q, Du X, Li M, Yuan M, Zhou Z (2012) Tetrahydroxystilbene glucoside improves the learning and memory of amyloid-beta((1)(−)(4)(2))-injected rats and may be connected to synaptic changes in the hippocampus. Can J Physiol Pharmacol 90(11):1446–1455. doi:10.1139/y2012-121

Zhu Z, Li C, Wang X, Yang Z, Chen J, Hu L, Jiang H, Shen X (2010) 2,2′,4′-trihydroxychalcone from Glycyrrhiza glabra as a new specific BACE1 inhibitor efficiently ameliorates memory impairment in mice. J Neurochem 114(2):374–385. doi:10.1111/j.1471-4159.2010.06751.x

Zhu KY, Xu SL, Choi RC, Yan AL, Dong TT, Tsim KW (2013) Kai-xin-san, a Chinese herbal decoction containing ginseng radix et rhizoma, polygalae radix, acori tatarinowii rhizoma, and poria, stimulates the expression and secretion of neurotrophic factors in cultured astrocytes. Evid Based Complement Alternat Med 2013:731385. doi:10.1155/2013/731385

Tea Polyphenols in Parkinson's Disease

6

Mario Caruana and Neville Vassallo

Abstract

Parkinson's disease (PD) is a common motor neurodegenerative disorder with multifactorial etiology that is an increasing burden on our aging society. PD is characterized by nigrostriatal degeneration which might involve oxidative stress, α-synuclein (αS) aggregation, dysregulation of redox metal homeostasis and neurotoxicity. Although the exact cause remains unknown, both genetic and environmental factors have been implicated. Among the various environmental factors tea consumption has attracted increasing interest, as besides being one of the most consumed beverages in the world, tea contains specific polyphenols which can play an important role in delaying the onset or halting the progression of PD. Green and black teas are rich sources of polyphenols, the most abundant being epigallocatechin-3-gallate (EGCG) and theaflavins. There is now consistent mechanistic data on the neuroprotective and neuroregenerative effects of tea polyphenols, indicating that they do not just possess anti-oxidant or anti-chelating properties but may directly interfere with aggregation of the αS protein and modulate intracellular signalling pathways, both *in vitro* and in animal models. EGCG in green tea has been by far the most studied compound and therefore future investigations should address more the effects of other polyphenols, especially theaflavins in black tea. Nevertheless, despite significant data on their potential neuroprotective effects, clinical studies are still very limited and to date only EGCG has

M. Caruana, Ph.D. (✉) • N. Vassallo, M.D., Ph.D.
Department of Physiology and Biochemistry,
University of Malta, Msida, Malta
e-mail: mario.caruana@um.edu.mt

N. Vassallo (ed.), *Natural Compounds as Therapeutic Agents for Amyloidogenic Diseases*,
Advances in Experimental Medicine and Biology 863, DOI 10.1007/978-3-319-18365-7_6

reached phase II trials. This review collates the current knowledge of tea polyphenols and puts into perspective their potential to be considered as nutraceuticals that target various pathologies in PD.

Keywords
Parkinson's disease • Alpha-synuclein • Tea • Epigallocatechin-gallate • Theaflavins • Neuroprotection

6.1 Introduction

Parkinson's disease (PD) is the most common movement disorder and after Alzheimer's Disease (AD), the second most frequent progressive neurodegenerative disease (Toda 2007). The prevalence of PD worldwide ranges from 0.5 to 4 % among people aged 65 years or older. This figure is expected to rise significantly with the accelerated aging of human society (de Lau and Breteler 2006). In fact, it was predicted that by 2030, the number of PD sufferers will reach 9.3 million (Dorsey et al. 2007). PD is a debilitating disorder with varying patterns of degeneration in the dopaminergic and nondopaminergic neuronal systems (Braak et al. 2004). The major symptoms of PD include muscular rigidity, uncontrollable resting tremor, bradykinesia or akinesia, and impaired postural reflexes (Jankovic 2008). PD is distinguished from other forms of parkinsonism by the presence of Lewy bodies (LBs) and Lewy neurites (LNs), which are juxtanuclear and neuritic ubiquitinated protein aggregates composed predominantly of the presynaptic protein α-synuclein (αS) (Shults 2006). The etiology of PD in most patients remains unknown. It is assumed that both genetic and environmental factors with complex interactions are responsible for the development and progression of the disease (Fig. 6.1; Logroscino 2005).

The pathogenesis and relative selectivity of death of dopaminergic neurons in the substantia nigra (SN) *pars compacta* remains to be clarified (Kazantsev and Kolchinsky 2008). Various pathogenic mechanisms have been proposed through which dopamine-releasing neurons may be damaged in PD. These include deficiency in mitochondrial respiratory chain function (Fukae et al. 2007), apoptosis (Hartmann and Hirsch 2001), transition metal accumulation (Barnham and Bush 2008), oxidative stress (Friedman and Galazka-Friedman 2001), deficiency in the xenobiotic mechanism (Ramsden et al. 2001), inflammation (McGeer et al. 2001), and abnormal protein handling, aggregation and misfolding (Skovronsky et al. 2006). The most favoured ones are oxidative stress due to an increased production of reactive oxygen species (ROS), and cell toxic effects of αS protein aggregation and deposition, both finally leading to neuronal cell death by apoptosis (Soto 2003). Regardless of the cause of neuronal death, the plasticity of the *pars compacta* is very robust; symptoms do not appear until 50–80 % of SN dopaminergic neurons have died. Therefore, it is not surprising that diagnosis in the early course of disease is more than rare (Jankovic 2008).

The results of studies on twins suggested that genetic factors are important in early-onset PD cases while environmental factors play a predominant etiologic role in late-onset PD patients, thus implying the importance of non-genetic factors (Tanner et al. 1999; Wirdefeldt et al. 2004). Environmental factors such as coffee drinking and smoking have been demonstrated to lower the risk of PD (Hernan et al. 2002; Costa et al. 2010; Wirdefeldt et al. 2011). The effects of tea consumption on PD risk are currently the subject of considerable scientific debate as tea components, such as polyphenols, caffeine, and theanine, have been demonstrated to be neuroprotective in PD (Tan et al. 2008; Quintana et al. 2009). The benefits of tea drinking are of relevance to PD as tea is one of the main contributors of dietary polyphenols in Western countries due to its regular consumption (Erdman et al. 2007).

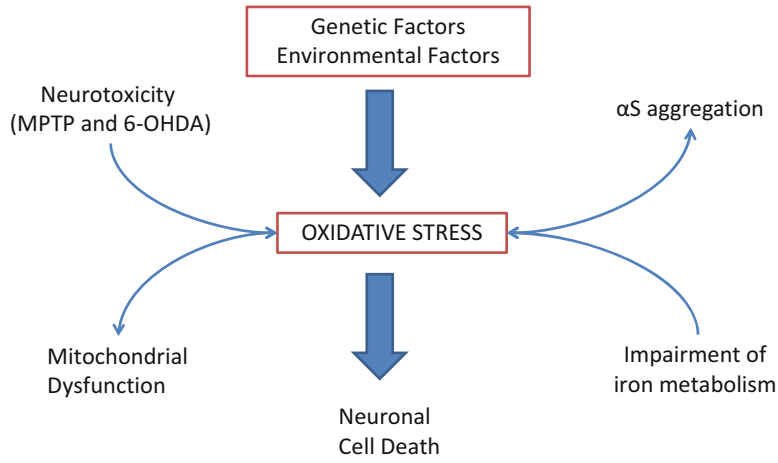


Fig. 6.1 Factors that contribute to oxidative stress and ultimately neuronal cell death in PD (*MPTP* N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, *6-OHDA* 6-hydroxydopamine, *αS* alpha-synuclein)

Thus, any evidence of the neuroprotective effects of polyphenols on PD could have a significant impact on public health. The purpose of this chapter is to provide a concise review of the most recent scientific evidence from epidemiological, experimental and clinical studies on the crucial role green and black tea polyphenols may have in the prevention and treatment of PD.

6.2 Polyphenolic Components of Tea

Tea has been consumed as a beverage for well over 2,000 years, and its worldwide consumption is perhaps second only to water. The term 'tea' refers to the dried leaves of the plant *Camellia sinensis*, an evergreen shrub of the *Theaceae* family. The three principal varieties of tea are generally categorized by the process used in their manufacture: (i) fermented (oxidized) black tea (78 %, mainly consumed in Western Europe, the United States of America, Australia, and some Asian countries); (ii) unfermented (non-oxidized) green tea (20 %, mainly consumed in China, Japan, and India); and (iii) semi-fermented (semi-oxidized) oolong tea (2 %, consumed in south-eastern China and Taiwan) (Fig. 6.2; Graham 1992; Balentine et al. 1997). Another commonly used tea is the so-called 'herbal tea'. Herbal tea is made from any of a number of a variety of plants and herbs, and therefore, cannot technically be considered a true type of tea.

While tea consists of over 2,000 different chemical substances such as methylxanthine, caffeine, lipids, amino acids, mineral substances and volatile compounds, polyphenols are the most abundant (Wheeler and Wheeler 2004). Polyphenols are a diverse class of plant secondary metabolites and more than 8,000 polyphenolic compounds have currently been identified (Porat et al. 2006; Stevenson and Hurst 2007). Polyphenols are classified into different groups depending on the number of phenol rings and the chemical groups attached to the rings. They are characterised by a polyphenol structure, which generally consists of two aromatic rings (2-phenyl-1,4-benzopyrone) each containing at least one hydroxyl group, which are connected via a three-carbon bridge and become part of a six-member heterocyclic ring (Fig. 6.3; Beecher 2003; Porat et al. 2004; D'Archivio et al. 2007). Polyphenols can be divided into two main groups, the flavonoids and the non-flavonoids, with the flavonoids making up the largest and most important single group of polyphenols present in tea (Vassallo 2008). Black and green teas both contain similar amount of flavonoids, however they differ in their chemical structure. Green teas contain more of the simple flavonoids called flavanols (known also as catechins or flavan-3-ols). The principal four flavanols found in green tea are epicatechin (EC), epicatechin 3-gallate (ECG), epigallocatechin (EGC) and epigallocatechin-3-gallate (EGCG), where the latter is the most abundant (Rietveld

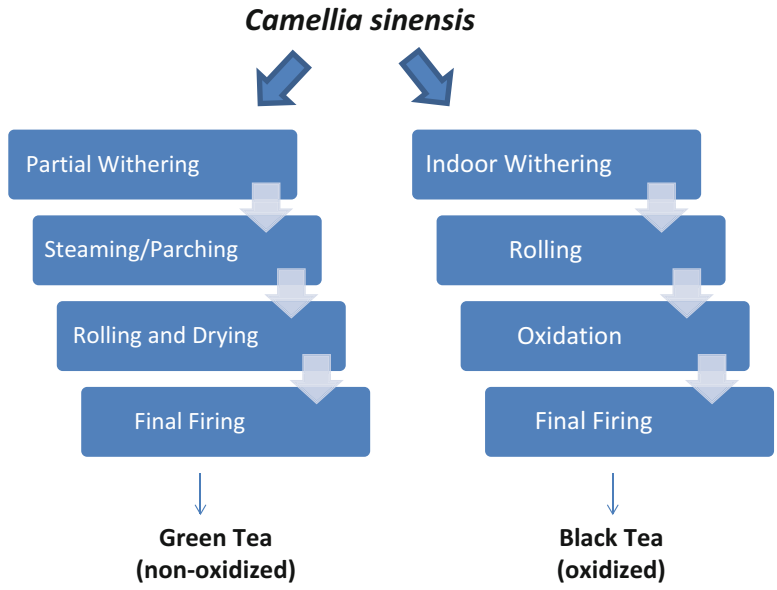


Fig. 6.2 Green and black tea processing. Tea is produced when freshly picked leaves are steamed, rolled and dried. Tea leaves contain polyphenol oxidase enzymes in separate layers of the leaf. When tea leaves are rolled or broken during industry manufacture, polyphenols known as flavanols (catechins) come in contact with polyphenol oxidase, resulting in their oxidation and the formation of flavanol dimers and polymers known as theaflavins and thearubigins. Tea leaves destined to become black tea are rolled and allowed to ferment (oxidize), resulting in relatively high concentrations of theaflavins and thearubigins and relatively low concentrations of flavanols. Green tea is withered and then steamed to inactivate polyphenol oxidase. Consequently, green tea contains relatively high concentrations of flavanols and low concentrations of theaflavins and thearubigins (Graham 1992; Balentine et al. 1997)

and Wiseman 2003). The oxidisation that the leaves undergo to make black tea converts these simple flavonoids to the more complex varieties called theaflavins and thearubigins (Khokhar and Magnusdottir 2002). The chemical composition of tea (Table 6.1) varies with the variety of plant and age of the leaf, the conditions under which it is grown, climate, season, and local agricultural practices (Aherne and O'Brien 2002).

Research interest in the benefits of tea drinking stems primarily from the presence of polyphenols which are believed to be the major component that provide health benefits (McKay and Blumberg 2002; Erdman et al. 2007). One cup of tea (2 g of tea leaves infused in hot water for 1–3 min) will provide 0.15–0.2 g of flavonoids. As little as 2–3 cups/day of tea will therefore supply a significant contribution to the total flavonoid intake in most individuals, which is estimated to average 1 g per day (Frei and Higdon 2003). In fact, it was estimated that black tea contributes 60–84 % of dietary flavonoids in Western populations (Hertog et al. 1993; Chun et al. 2007) and it has been reported that flavonoid intakes in tea consumers are twenty times greater than in non-tea consumers (Song and Chun 2008). This intake is higher than all other known dietary anti-oxidants, estimated to be around ten times higher than the daily intake of Vitamin C, and 100 times higher than that of Vitamin E and carotenoids (Scalbert and Williamson 2000). As the total polyphenol content of green and black teas is similar, it can be assumed that the impact on plasma levels post-consumption remains fairly the same (Rietveld and Wiseman 2003).

6.3 Epidemiological Studies on Tea Consumption and PD

In the 1980s, PD prevalence was found to be low in Asian countries when compared to Europe and North America, which had significantly higher

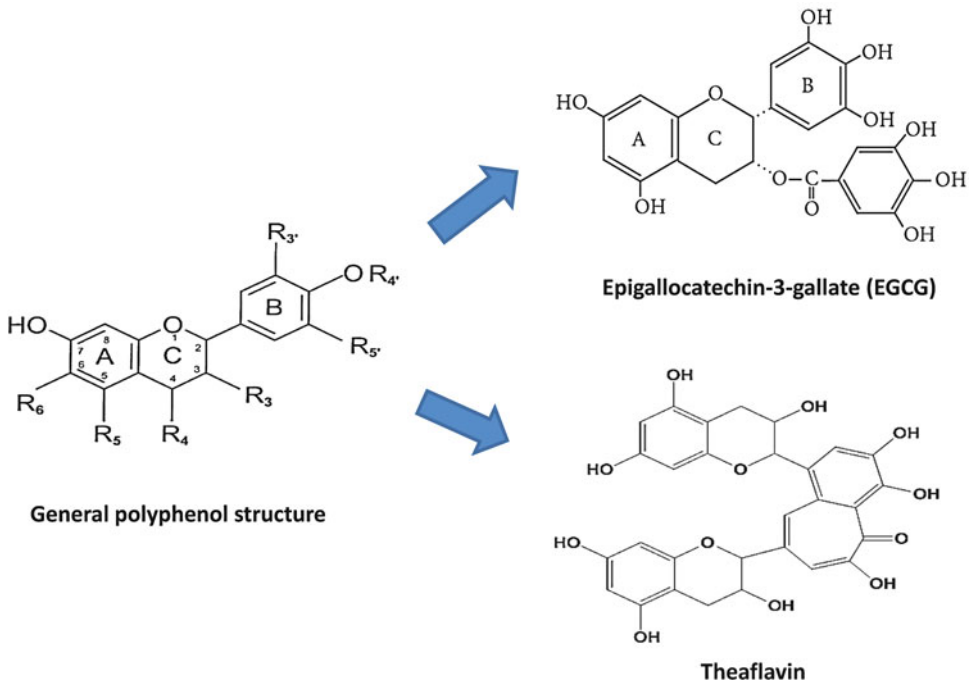


Fig. 6.3 General structure and numbering pattern for polyphenols. This figure shows the general structure and numbering pattern for common polyphenols. Every flavonoid subclass has its own unique linkages, unsaturation positions and functional groups. For most food flavonoids, R4′ = H, R5 = OH and R6 = H. Individual flavonoids within each subclass are characterised by unique functional groups at R3, R3′, and R5′. Chemical structures of epigallocatechin-3-gallate (EGCG) and theaflavin in green and black tea are shown. EGCG contains three heterocyclic rings (*A*, *B*, *C*) and the free radical scavenging property of EGCG is attributed to the presence of a trihydroxyl group on the B-ring and the gallate moiety at the 3′ position in the C-ring. Theaflavin is the polymeric form of EGCG

rates (Li et al. 1985; Zhang and Román 1993). Apart from genetic factors, dietary habits like green tea consumption, which is more consumed by the Chinese population when compared to Caucasian, could explain this attribute (Pan et al. 2003; Gao et al. 2012). Due to this possible link, in recent years, there were more studies devoted to exploring the effects of tea consumption on PD risk. Three case–control studies (in the US, Hong Kong and Singapore) and a cohort study of male health professionals in the US have all reported an inverse association between tea drinking and PD risk (Chan et al. 1998; Checkoway et al. 2002; Tan et al. 2003). One study found such an effect for men but not for women (Ascherio et al. 2001). On the other hand, a hospital based case-control study in France reported tea consumption to be a paradoxically risk factor for PD (Preux et al. 2000).

The authors attributing the protective effect of tea suggested caffeine as the main contributor. Similarly, a biologic effect of caffeine was suggested for a positive association of tea drinking and PD in a prospective study of over 29,000 Finnish adults for 13 years (Hu et al. 2007). In another prospective study of 63,000 Chinese adults, black tea showed an inverse association with PD risk, although this time the link was not confounded by total caffeine intake or tobacco smoking (Tan et al. 2008). Surprisingly in this study, green tea consumption after adjustment for cigarette smoking and total caffeine consumption was unrelated to PD risk. The authors speculated that the protective effect of black tea may be mediated via an estrogen-related pathway. This was based on what they had reported earlier that among the women in their study cohort, levels of circulating estrogens were highest in

Table 6.1 Average values for the different constituents present in green and black tea

	Black tea	Green tea
Catechins	3–10	30–42
Theaflavins	3–6	Negligible
Thearubigins	12–18	Negligible
Flavonols	6–8	5–10
Theogallin	Negligible	2–3
Phenolic acids (caffeic acid)	10–12	1–2
Theanine	Negligible	4–6
Other amino acids	13–15	4–6
Methylxanthines	8–11	7–9
Carbohydrates	15	10–15
Protein	1	Negligible
Minerals	10	6–8
Volatiles	<0.1	0.02

The values will differ dependent on the variety of leaf, growing environment, manufacturing, particle size of ground tea leaves and infusion preparation (Graham 1992; Harbowy and Ballentine 1997; Wang and Helliwell 2001; Astill et al. 2001)
Values reflect % weight of extract solids

regular black tea drinkers, intermediate in non-tea drinkers, and lowest in regular green tea drinkers; these differences were dose-dependent and significant (Wu et al. 2005).

It was suggested that the average 1.2 l of green tea consumed daily by many people in Asia offers sufficient anti-oxidants of the polyphenolic EGCG, and in turn reduces or cures diseases with an inflammatory component, together with improving neurologic and psychological health (Sumpio et al. 2006). In terms of a dose-response relationship, only few studies have stratified their results according to the number of cups of tea consumed daily. One study showed a dose-dependent protective effect of PD in tea consumers with an odds ratio (OR) of 0.48 for daily consumption of a cup of tea versus OR of 0.27 for daily consumption of two or more cups of tea (Fall et al. 1999), whilst another study showed a similar effect in coffee and tea consumers (Tan et al. 2003). In the latter study it was concluded that one unit of coffee or tea (three cups per day for 10 years) would lead to a 22 and 28 % risk reduction of PD, respectively. On the contrary, another study could not demonstrate such dose-dependent protective effect in their hospital based case-control study (Paganini-Hill 2001). More evidence was provided by an Israeli study of 278 PD patients whose motor symptoms appeared to be delayed by 7.7 years ($p < 0.01$) when they drank more than three cups of tea per day (Kandinov et al. 2009). Two recent systematic reviews showed that tea drinking can lower the risk of PD, but no apparent dose-response relationship was found as would be expected (Quintana et al. 2009; Li et al. 2012). The latter may arise from the fact that black tea and green tea differ markedly in the nature of their polyphenols and only few studies reported stratified results according to the types of tea. It is also important to note that the contents of the bioactive compounds in tea may fluctuate because of differences in producing areas, materials, and manufacturing (Crozier et al. 2009). While the addition of milk to tea does not seem to interfere with flavonoid absorption or activity (Hollman et al. 2001), it is not obvious if other factors do – such as the frequency and timing of tea intake in relation to meals, the addition of sucrose or lemon, and variations in gut microflora. Therefore, although a positive association has turned up repeatedly in epidemiological studies between tea and PD, a clear biologic basis for this phenomenon has yet to be identified.

When reviewing the literature, the strongest and most consistent environmental associations were those between cigarette smoking, coffee/tea drinking, and a reduced risk of PD as noted in several US and European populations (Tanner et al. 2002; Hernan et al. 2002; Ritz et al. 2007; Hancock et al. 2007; Saaksjarvi et al. 2008). However, the strength of the evidence for the described inverse associations seems to be weaker for tea than for smoking or coffee drinking. The precise reasons for this are not known, although tea has not been investigated in relation to PD risk as extensively or explicitly as coffee has – perhaps because consumption of coffee is far more prevalent in North America and Europe, where most research on PD has been undertaken.

Moreover, the selection of patients and the type of control groups, for example the inclusion of patients with preclinical stage of PD, may result in conflicting results (Schrag et al. 2002). It is unclear from epidemiological studies whether the active ingredient/s mediating this neuroprotective effect in tea is actually caffeine or the polyphenols in tea. In most cases, this work can only be conducted in experimental designs not least because practical and ethical constraints limit such research in humans.

6.4 Neuroprotective Actions of Tea Polyphenols in PD

6.4.1 Tea Polyphenols and *In Vitro* Studies

Numerous *in vitro* studies have clearly demonstrated that specific tea polyphenols might contribute to prevent PD pathology and act towards neuroprotective capacities (Levites et al. 2002a; Bastianetto 2002; Bastianetto and Quirion 2004). Cell culture studies have demonstrated that flavanols reduced damage produced by hydrogen peroxide (H_2O_2), 4-hydroxynonenal, rotenone, and 6-hydroxydopamine (6-OHDA) in primary rat mesencephalic cultures, as shown by increases in cellular viability and [^{3}H] dopamine uptake (Mercer et al. 2005; Vauzour et al. 2008). Other *in vitro* studies demonstrated that EGCG is able to rescue and reduce viability of neuroblastoma SH-SY5Y cells when administered up to 3 days after long-term serum starvation, a model of apoptotic damage (Reznichenko et al. 2005). As reviewed elsewhere, polyphenolic compounds provide neuroprotective effects through a variety of biological actions such as anti-oxidant, anti-chelating, anti-aggregating, anti-inflammatory, anti-carcinogenic, anti-viral, anti-microbial and anti-clotting activities (Scalbert et al. 2005; Ramassamy 2006; Rahman et al. 2007; Moon and Shibamoto 2009; Obrenovich et al. 2010; Albani et al. 2010; Choi et al. 2012). With regards to specific tea polyphenols, the most important plausible mechanisms cited that may be exhibiting neuroprotective effects in PD are: (i) anti-oxidant and anti-chelating activities; (ii) inhibition of αS aggregation; and (iii) modulation of cell signalling pathways (Pan et al. 2003; Higdon and Frei 2003; Amit et al. 2008), which will be reviewed in the next sections.

6.4.1.1 Anti-oxidant and Iron-Chelating Activity

Substantial evidence of the potent anti-oxidant effects of the main tea polyphenols (flavanols and theaflavins) comes from *in vitro* studies, where they were shown to: (i) directly scavenge reactive oxygen (ROS) and nitrogen oxygen (NOS) species; (ii) inhibit 'pro-oxidant' enzymes, such as nitric oxide synthase, xanthine oxidase, cyclooxygenases and lipoxygenases; (iii) inhibit redox-sensitive transcription factors such as nuclear factor-κB and activator protein-1; (iv) induce phase II and anti-oxidant enzymes such as glutathione S-transferases and superoxide dismutases; and (v) bind and chelate excess of divalent metals such as iron (Fe^{2+}) and copper (Haenen et al. 1997; Nakagawa and Yokozawa 2002; Higdon and Frei 2003; Stevenson and Hurst 2007; Aron and Kennedy 2008; Mandel et al. 2008; Perron and Brumaghim 2009; López-Lázaro 2009). The oxygen radical absorbance capacity (ORAC) assay has demonstrated that both green and black tea have much higher capacity against free radicals than vegetables, for instance garlic and spinach (Cao et al. 1996).

The capacity of polyphenols to act as anti-oxidants is dependent upon their molecular structure, the position of hydroxyl groups, and other substitutions in their chemical structure (Tsao 2010). Although the oxidisation process modifies the type of flavonoids present, the total level and their overall anti-oxidant activity, is similar in both teas (Leung et al. 2001; Luczaj and Skrzydlewska 2005). EGCG has an important anti-oxidant and iron-chelating function and this could be attributed to the 3′,4′-dihydroxyl group in the B-ring, as well as the gallate group which may neutralise Fe^{2+} to form redox-inactive iron, thereby protecting cells against oxidative damage (Fig. 6.3; Kumamoto et al. 2001). In addition, the feature of tea polyphenols as potent chelators

of transitional metals, such as iron and copper, is owed to the OH at position 3′ of the C-ring, the OH at positions 3′ and 4′ of the B-ring, or the three OH groups present in the gallol moiety of some polyphenols, such as EGCG and ECG (Nanjo et al. 1996). In a recent study examining the differential potency of a series of polyphenols to prevent DNA damage caused by Fe^{2+} and H_2O_2, it was found that among the 12 phenolic compounds tested, EGCG was the most potent, inhibiting over 90 % of the iron-mediated DNA break (Perron et al. 2008). By correlating the pK_a and IC_{50} values of phenolic compounds for inhibition of Fe^{2+}/H_2O_2-induced neurotoxicity, it was suggested that the binding of the polyphenols to iron was essential for their anti-oxidant activity (Perron et al. 2010). In one experiment performed on rat brain tissue, it was shown that lipid peroxidation was enhanced by iron ascorbate but inhibited in brain mitochondria by both black and green tea extracts (Jeong et al. 2004).

The anti-oxidant and metal-complexing properties of tea polyphenols may be of significance in the treatment of PD, since oxidative stress and accumulation of Fe^{2+} at brain areas associated with neurodegeneration have been clearly demonstrated (Zecca et al. 2004). The anti-oxidant activity could protect the dopaminergic system against free radicals, anion superoxide, lipid free radicals and hydroxyl radicals, together with neurotoxic apoptosis induced by hydroxydopamine in the cell (Weinreb et al. 2004). Also, at the central nervous system (CNS) level it may inhibit the peroxidation and lipid accumulation of Fe^{2+} compounds, and this could be the main mechanism for neuroprotection (Soto-Otero et al. 2000; Pan et al. 2004; Levites et al. 2002b). Nevertheless, it is currently more accepted that neuroprotective effects of tea polyphenols are only partly attributed to the free radical scavenging or metal chelating properties and that other properties such as targeting of specific signalling pathways and interaction with specific proteins, including αS, contribute to neuroprotection (Kaur et al. 2003; Masuda et al. 2006; Ramassamy 2006; Vafeiadou et al. 2007; Weinreb et al. 2010).

6.4.1.2 Inhibitory Activity on α-Synuclein Aggregation

Ample evidence suggests that disturbance of neuronal membranes by the soluble oligomers of the protein αS is a likely first step in the pathophysiological cascades of PD, where partial aggregated and oligomerized intracellular αS was shown to be cytotoxic and synaptotoxic (Periquet et al. 2007; Selkoe 2008). A considerable amount of scientific data shows that a possible neuroprotective characteristic of polyphenolic compounds is exerted through anti-aggregating properties (Caruana and Vassallo 2011). In relation to PD, such properties were initially tested *in vitro* on the inhibition of the assembly of αS into filaments/fibrils (Conway et al. 2001). For example, tea polyphenols such as EGCG and black tea extract, inhibited wild-type (WT) αS filament assembly and were also found to disaggregate preformed fibrils (Zhu et al. 2004; Porat et al. 2006; Masuda et al. 2006; Ono and Yamada 2006; Meng et al. 2010; Grelle et al. 2011). Recently, EGCG efficiently inhibited fibril formation of αS (Ehrnhoefer et al. 2008; Bae et al. 2010) and also transformed large αS fibrils into smaller non-toxic, amorphous protein aggregates (Hudson et al. 2009; Bieschke et al. 2010). Biophysically, EGCG was postulated to directly bind to unfolded polypeptide chains via hydrogen bonds and hydrophobic peptide backbone interactions, and inhibit beta sheet formation which is the early event in the amyloid formation cascade (Wang et al. 2010). Interestingly, this effect was evident only with flavanols carrying a gallate moiety with a high affinity for metals, such as ECG and EGCG. In this regard, another study showed that only gallate forms of flavanols were able to protect hippocampal cells against amyloid-induced toxicity (Bastianetto et al. 2006). Thus, it is possible that the anti-fibrillogenesis action of polyphenols would also result from an iron-complexing radical scavenging-mediated action.

Since it is hypothesised that small αS oligomers, rather than fibrils, may be the primary toxic species, it was also shown that polyphenolic compounds inhibit and destabilise early stage aggregates (Caruana et al. 2011). Interestingly *in vitro* studies have shown that

the anti-oxidant activities of such polyphenols are not likely to be directly involved in the inhibition progress (Zhu et al. 2004; Johnston and Brotchie 2004; Caruana et al. 2011). Specific structural features, rather than broad biochemical characteristics, determine the anti-aggregation effects of polyphenols. In fact, flavonoids with three vicinal hydroxyl groups exhibited enhanced inhibitory effects on αS aggregation (Meng et al. 2009; Berhanu and Masunov 2010; Caruana et al. 2011). Essentially, a polyphenolic inhibitor, with its polyaromatic nature is able to use aromatic recognition elements to bind the monomer/oligomer, whilst utilizing the vicinyl hydroxyl groups to electrostatically block the progress of the self-assembly process (Gazit 2002; Porat et al. 2006).

The lipophilicity of biologically active compounds is usually one of their most important pharmacological features, and interactions with membranes play an essential role in their biological activity (Hendrich 2006). It is well established that small αS oligomers can interact with and perturb membranes, thereby leading to cell death (Lashuel et al. 2002; Quist et al. 2005; Winner et al. 2011). Similarly, polyphenolic compounds, including tea polyphenols, interact with and alter lipid membranes (Blazovics et al. 2000; Oku et al. 2003; Chen et al. 2011; Duchnowicz et al. 2012; Sharma et al. 2012). Indeed, it was revealed that EGCG inhibits amyloid formation less efficiently at phospholipid interfaces than in bulk solution (Engel et al. 2012). Hence, it is relevant to know how polyphenolic compounds directly effect lipid membranes and how efficiently they can inhibit αS aggregation specifically at the phospholipid membrane interface. Furthermore, it has been established that tea flavanols can adsorb to membranes through associations with the polar headgroups of phospholipids and could protect the integrity of lipid bilayer from disrupting agents (Verstraeten et al. 2003; Sirk et al. 2008, 2011). Such polyphenol-lipid interactions may provide a level of protection for the bilayer from the αS oligomers (or/and monomer aggregation at the membrane surface), contributing to preserve the structure and function of biological membranes. Not many studies have to date examined the interaction of polyphenols with neuronal membranes and their protective effect on αS-induced membrane dysfunction. For example, black tea extract (80 % theaflavin) was found to strongly protect against membrane perturbation induced by aggregated WT and mutant αS (Caruana et al. 2012). Moreover, black tea extract inhibited permeation of mitochondrial membranes by αS oligomers (Camilleri et al. 2013). The importance of mitochondria in the pathogenisis of PD and the cytoxicity of oligomeric αS is extensively reviewed elsewhere, and tea polyphenols have been capable of protecting such insults (Büeler 2009; Camilleri and Vassallo 2014; Caruana and Vassallo 2014). Therefore, it is important that the intracellular effects of polyphenols at the membrane level are known, enhancing our understanding of the pharmacological and therapeutic activities of such bioactive compounds.

6.4.1.3 Modulation of Intracellular Signaling Pathways

While there has been a historical spotlight on the anti-oxidant properties of tea polyphenols, there is a general consensus that such flavonoids and their corresponding *in vivo* metabolites may also exert modulatory actions intracellularly through direct action on various signalling pathways in a concentration-dependent manner (Mandel et al. 2004; Williams et al. 2004; Ramassamy 2006; Campos-Esparza and Torres-Ramos 2010). Various inhibitory or stimulatory actions of tea polyphenols on these pathways have been studied, including phosphoinositide 3-kinase (PI3K), protein kinase B (Akt/PKB), protein kinase C (PKC), and mitogen-activated protein kinase (MAPK) (Levites et al. 2002b; Vauzour et al. 2007). The effects of tea polyphenols on signaling pathways in relation to PD will be reviewed briefly.

Most studies related to modulation of cell signalling pathways have been carried out on flavanols found in green tea, but not theaflavins. Table 6.2 summarizes cell signalling pathways targeted by different tea polyphenols. Protein kinase C (PKC) is the target of many flavonoids for providing survival signalling. For example, EGCG at a dose of 2 mg/kg body weight markedly in-

Table 6.2 Cell signalling pathways targeted by different tea polyphenols in neuronal cell lines

Tea polyphenol	Activation (+) Deactivation (−)	Signalling pathways	Neuroprotection	References
EGCG; ECG	–	MAPK	Prevent apoptosis, oxidative stress and endothelial barrier dysfunction, improve mitochondrial functions	Huang et al. (2007), Hwang and Yen (2009), Yang et al. (2010)
EGCG; ECG	–	JNK	Prevent oxidative stress and apoptosis	Choi et al. (2005), Huang et al. (2007)
EGCG; ECG; EC	+	ERK1/2	Prevent oxidative stress and apoptosis	Schroeter et al. (2007)
EGCG, EC	+	PKB	Anti-oxidant defence, prevent oxidative stress-induced apoptosis, stimulate eNOS activity, regulate mitochondrial function	Schroeter et al. (2007), Vauzour et al. (2007), Na et al. (2008), Hwang and Yen (2009), Yang et al. (2010)
EGCG	+	PKC	Neuroprotective	Levites et al. (2002b)
EGCG	+	PI3K	Prevents oxidative stress-induced apoptosis, protects oxidative damage, stimulate eNOS activity	Levites et al. (2002b), Yang et al. (2010), Xi et al. (2012)

The activation of ERK, Akt/PKB, PI3K, and PKC is important to improve cell survival, and the down-regulation of P38 and JNK in preventing apoptosis. The activation of signalling pathways is shown as (+) while down-regulation of signalling pathways by tea polyphenols is shown as (−)
MAPK p38 mitogen-activated protein kinase, *PKB* protein kinase B, *ERK* extracellular signal-regulated protein kinase, *JNK* c-Jun N-terminal kinase, *PI3K* phosphatidylinositol-3 kinase, *PKC* protein kinase C, *eNOS* endothelial nitric oxide synthase, *EC* (−)epicatechin, *ECG* (−) epicatechingallate, *EGCG* (−)-epigallocatechingallate

creased PKC in the membrane and cytosolic fractions of mice hippocampus. EGCG restored the reduced PKC and extracellular signal-regulated kinase (ERK1/2) activities caused by 6-OHDA toxicity, and protected against neuronal apoptosis (Levites et al. 2002a). EGCG is also involved in rapid PKC-mediated degradation of the Bcl-2-associated death promoter (Bad) by the ubiquitin proteosome system, thus neutralizing their pro-apoptotic function (Calixto et al. 2004).

Tea polyphenols have been shown to interact with ERK, c-Jun N-terminal kinase (JNK) and p38 pathways of the mitogen-activated protein kinases (MAPKs). EC was shown to modulate protein kinase signalling pathways, depending on the concentration of the compound administered (Schroeter et al. 2007). EC stimulated ERK1/2 and phosphoinositide-3-kinase (PI3K)-dependent cAMP response element-binding protein (CREB) phosphorylation at lower concentrations of 100–300 nM but this effect was no longer apparent at the higher concentration of 30 μM. These dose-dependent effects may be important to explain the anti- versus pro-oxidant actions of the tea polyphenols. EC also stimulated ERK and Akt phosphorylation. A 15-min exposure of EC increased the mRNA levels of the glutamate receptor subunit (GluR2) by 60 %, and resulting in increased GluR2 protein. This suggests that EC has the potential to increase CREB-regulated gene expression and increase GluR2 levels and thus modulate neurotransmission, plasticity, and synaptogenesis (Schroeter et al. 2007). EGCG inhibited H_2O_2-induced phosphorylation of JNK and p38 MAPK pathway after a 60-min exposure. EGCG also inhibited H_2O_2-induced caspase-3 activation at concentrations between 1 and 50 μM (Choi et al. 2005). Thus, MAPK-related signalling may regulate expression of apoptotic genes, preventing apoptosis, and promoting cell survival. Another observation demonstrates that EGCG at concentrations between 5 and 25 μM

inhibits angiotensin II-induced endothelial stress fibre formation and increased permeability via inactivation of p38/heat shock protein 27 (HSP27) pathway and suggests that EGCG may protect against endothelial barrier dysfunction and injury (Yang et al. 2010). EGCG treatment also increased the nuclear accumulation, anti-oxidant response element (ARE) binding, and transcriptional activity of nuclear factor erythroid 2-related factor 2 (Nrf2). Furthermore, EGCG activated Akt and ERK1/2. These findings suggest that Nrf2 mediates EGCG-induced expression of some representative anti-oxidant enzymes, possibly via Akt/PKB and ERK1/2 signalling, which may provide the cells with acquired anti-oxidant defence capacity to survive the oxidative stress (Na et al. 2008). In addition to MAPKs pathway, flavonoids and their metabolites have been also shown to modulate cell survival signalling due to their interaction with the PI3K/Akt pathway (Kyoung et al. 2010). The PI3K/Akt pathway is one of the strongest intracellular pro-survival signalling systems. EGCG activated Akt and ERK1/2 signalling cascade in MCF10A cells (Na et al. 2008). This effect was mediated partially via the activation of the downstream pAkt and pBad pathways.

EGCG and other flavanols found in green tea are by far the most intensely investigated, with no studies being reported on other black tea constituents. Thus, besides its free radical scavenging, iron chelating, and anti-aggregating properties, EGCG can exert its action on different sites of the apoptotic pathways, including altering the expression of anti- and pro-apoptotic genes. These studies further implicate that green tea extract may also exert protection through controlling calcium homeostasis, activation of MAPK, PKC, anti-oxidant enzymes and survival genes, thus potentially preventing progression of PD.

6.4.2 Tea Polyphenols and *In Vivo* Models of PD

A number of studies have reported on the protective effects of tea polyphenols against brain damage in various animal models of PD (Dajas et al. 2003; Mandel and Youdim 2004; Mandel et al. 2008). Studies have used either a single compound such as EGCG, or a complex mixture of extracts from tea (Mercer et al. 2005; Masuda et al. 2006; Weinreb et al. 2008; Chen et al. 2008). Green or black tea polyphenol extracts, as well as individual EGCG, attenuated striatal dopamine depletion and SN dopaminergic neurons loss when given chronically to mice, rats or monkeys treated with the parkinsonism-inducing neurotoxins, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 6-OHDA (Levites et al. 2001; Chaturvedi et al. 2006; Chen et al. 2014). One study concluded that EGCG does not protect against 6-OHDA-induced loss of nigrostriatal neurons in rats (Leaver et al. 2009). More recently, it was demonstrated that stand-alone polyphenols including EC, EGC, and EGCG protect, rescue and most importantly restore the impaired movement activity (climbing capability) induced by paraquat in *Drosophila* models of PD (Jimenez-Del-Rio et al. 2010). Significantly, these findings receive further support from a recent *in vivo* preclinical neurorescue/neurorestorative drug cocktail study, demonstrating that synergistically EGCG and rasagiline (whilst individually having no profound protective effect) almost completely restored nigrostriatal dopaminergic neuron degeneration caused by MPTP (Reznichenko et al. 2010). Therefore, in a combination therapy regime, EGCG may have the potential to complement the pharmacological activities of current drugs in PD (Chen et al. 2008). Table 6.3 summarizes the most relevant studies concerning the neuroprotective and neurorestorative activities of tea polyphenols in animal models concerning PD.

Understanding the *in vivo* effects of tea consumption is far from complete. Evidence that tea polyphenols are acting directly or indirectly as anti-oxidants *in vivo* exists, but is far more limited when compared to *in vitro* studies. Administration of green tea extract and, in one case, black tea extract, attenuated decreases in superoxide dismutase (SOD) activity caused by infection, ethanol or the carcinogen, 3-methylcolanthrene (Frei and Higdon 2003; Higdon and Frei 2003).

Table 6.3 Neuroprotective and neurorestorative activities of tea polyphenols in animal models of PD

PD model	Tea polyphenol and oral dose	Neuroprotection	References
6-OHDA rat	(i) Black tea extract (1.5 %) *ad libitum*	Improvement of spontaneous locomotion, striatal dopamine and anti-oxidant enzymes, prevention and rescue of SN dopaminergic neurons	Chaturvedi et al. (2006)
	(ii) Green tea (150 mg/kg/day)	Protected dopaminergic neurons and preserved the free radical scavenging capability of both the midbrain and the striatum.	Guo et al. (2007)
MPTP mice	(i) EGCG (2 and 10 mg/kg/day), or Green tea extract (0.5 and 1 mg/kg/day)	Prevented dopamine neuron loss and depletion in striatal dopamine and hydroxlase protein levels	Levites et al. (2001)
	(ii) EGCG (25 mg/kg/day), and green tea (5 ± 0.7 ml)	Prevented the loss of tyrosine hydroxylase (TH)-positive cells in the SN and of TH activity in the striatum, preserved striatal levels of dopamine and its metabolites	Choi et al. (2002)
	(iii) EGCG (5 mg/kg/day)	Rescue of striatal dopamine depletion and SN dopaminergic neurons loss-induced by MPTP	Reznichenko et al. (2010)
	(iv) Theaflavins (10 mg/kg/day)	Reduces oxidative stress, improves motor behaviour and expression of dopamine transporter and vesicular monoamine transporter 2 in striatum and SN	Anandhan et al. (2012a, b)
MPTP monkey	Green tea extract (40 mg/kg/day)	Alleviates motor impairments and dopaminergic neuronal injury in the SN, inhibition of MPTP-induced accumulation of neurotoxic αS oligomers in the striatum and other brain regions	Chen et al. (2014)

6-OHDA 6-hydroxydopamine, *MPTP* N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, *SN* substantia nigra

Another study showed that green tea consumption prevented decline in glutathione peroxidise, indicating a protection in age-related oxidative damage in the brain (Kishido et al. 2007). While green and black tea administration improved the resistance of lipoproteins to *ex vivo* oxidation in several animal models, the improvement was generally much less than that conferred by supplementation with other anti-oxidants. Such an observation raises the question of whether tea polyphenols are present in sufficient quantities *in vivo* to work through an anti-oxidant mechanism (Lotito and Frei 2006; Stevenson and Hurst 2007; Spencer et al. 2009). Some studies have shown that blood concentrations of polyphenols are not high enough to add significantly to the body's total anti-oxidant capacity (D'Archivio et al. 2007; Ghosh and Scheepens 2009). In fact, it was estimated that, after ingestion, up to 95 % of polyphenols undergo structural modification, that in turn may change the 'biological activities' of polyphenols as observed in the *in vitro* studies (Lotito and Frei 2006; Stevenson and Hurst 2007). In turn, there is evidence that polyphenols may be working through other mechanisms, for instance, by protecting endogenous anti-oxidant enzymes such as ascorbic acid in the human body against oxidation consequently improving the overall anti-oxidant level *in vivo* (Aron and Kennedy 2008). This indirect contribution to anti-oxidant effects requires polyphenols at concentrations much lower than would be essential for chemical anti-oxidant protection *in vitro* (Spencer et al. 2009). In other words, polyphenols may act beyond their anti-oxidant activity when not present at suitable concentrations to exert anti-oxidative effects (Saura-Calixto et al. 2007).

It is well known that tea polyphenols differ in their bioavailability and bioactivity. The rather poor bioavailability of EGCG needs to be considered when results obtained *in vitro* are extrapolated to situations *in vivo*. Most of the ingested EGCG is actually not absorbed in the blood, since absorption takes place in the small intestine and substantial quantities pass from the small to the large intestine where it undergoes further degradation by the action of local microbiota (Auger et al. 2008; Stalmach et al. 2009; Roowi et al. 2010). Bioavailability studies for EGCG indicate that peak plasma concentrations are reached after 1–2 h in healthy subjects with one oral dose (800 mg) in the morning after an overnight fasting period; these levels diminish gradually to undetectable levels within 24 h (Chow et al. 2005). The elimination half-life of EGCG is around 3.4 ± 0.3 h (Lee et al. 2002). It was argued that since green and black tea display similar anti-oxidant potential *in vivo*, despite containing different classes of polyphenols, it can be assumed that at least some of the thearubigins and theaflavins are absorbed (Leung et al. 2001; Rietveld and Wiseman 2003). However, the bioavailability of individual thearubigins and theaflavins has thus far not been directly evaluated in human studies. Nevertheless, although the bioavailability of tea flavonoids is low, repeated consumption of tea drinks resulted in a significant accumulation of flavanols in most body organs with relatively high peak plasma levels (Henning et al. 2008). The lack of a precise analytical method to estimate the presence of the more bioavailable flavanols in green tea compared to theaflavins and thearubigins in black tea *in vivo* may lead to an underestimation of the bioactivity of black tea when compared to green tea (Kumar and Pandey 2013). There has been extensive debate about whether the addition of milk to tea affects the bioavailability of flavonoids. Studies have clearly shown that plasma levels of polyphenols such as flavanols increased significantly after tea consumption and were unaffected by the addition of milk even when considering *in vivo* anti-oxidant potential (Kyle et al. 2007). Assuming that small micromolar quantities of tea polyphenols can exhibit bioactivity *in vivo*, current data suggests that long-term consumptions of tea can result in the absorption and retention of sufficient amounts of polyphenols to exert the required effects in plasma and tissues.

Despite the increasing amount of evidence favouring the bioavailability of polyphenols in the systemic circulation, less information is available regarding their ability to cross the blood-brain barrier (BBB) and reach the CNS (Williamson and Manach 2005; Crozier et al. 2009). Flavanols were shown to cross a cellular model of the BBB in a time-dependent and stereo-selective manner (Faria et al. 2011). Multiple animal models have demonstrated that EGCG and EC cross the BBB, reaching a concentration of 0.5 nmol/g in rat brain in the case of EGCG consumption (500 mg/kg) and to co-localise within the brain tissues independently of their route of administration (Nakagawa and Miyazawa 1997; Abd El Mohsen et al. 2002; Adachi et al. 2006). These findings suggest that tea polyphenols are potential biologically active nutrients for direct neuroprotective and neuromodulatory actions. Although the uptake and distribution of dietary polyphenols within the brain is somewhat documented, more uncertainty revolves around the dosage, absorption, metabolism, tissue distribution, and intracellular accumulation and excretion of such compounds. Thus future work is required to investigate this further (Schaffer and Halliwell 2012; Vauzour 2012).

6.5 Clinical Studies with Tea Polyphenols in PD

Despite numerous efforts in the search for disease-modifying therapies in PD, currently the only approved treatment, apart from rasagiline, are agents that target symptoms without modifying the actual pathophysiology of the disease (Olanow et al. 2009). A double-blind, randomized, placebo-control delayed clinical study to evaluate the safety, tolerability, and efficacy of green tea polyphenols in slowing disease progression in patients with early PD, was conducted by the Chinese Parkinson Study Group (CPSG); 410 untreated people with early

PD were enrolled at 32 Chinese Parkinson Study Group sites. Participants were randomized to 0.4, 0.8, or 1.2 g of green tea polyphenols daily or placebo in the first phase of the study, and at 6 months the placebo group switched to 1.2 g of green tea polyphenols daily for 6 more months. Although insomnia was slightly increased, it was found that green tea polyphenols were well tolerated and provided a mild symptomatic relief in early untreated PD (Chan et al. 2009). Data from Chow and colleagues also confirmed that a daily dose of 800 mg caffeine-free EGCG for 4 weeks is safe and well tolerated in healthy human subjects (Chow et al. 2003).

Nonetheless, to date clinical trials so far have failed to identify compounds such as tea polyphenols with compelling proof for disease-modifying properties. One important reason is the lack of a reliable biomarker that can be used to track disease progression (Gerlach et al. 2012). An urgent need for suitable biomarkers in PD together with well-designed controlled studies to assess a risk reduction of PD with tea polyphenols should be prioritized so as to support the evidence derived from *in vitro* and *in vivo* studies.

6.6 Conclusion

Tea is one of the most frequently consumed beverages in the world and its medicinal effects have a long, rich history. In this review we have shown that in the last decade there has been an extensive interest in tea polyphenols as a potential therapeutic agent in PD (Mandel and Youdim 2004; Spencer 2008). Indeed, there is convincing evidence to suggest that the consumption of green and black tea exerts a beneficial effect in reducing the risk of PD, due to its polyphenolic content which exhibits numerous biochemical activities. From the epidemiological data reviewed, it was determined that the dose for the daily intake of tea should be around 2–3 cups/day, in order to induce neuroprotection. The observed beneficial effect, mostly from case–control studies, of tea drinking should now be investigated further in large prospective cohort studies.

There seems to be a consensus that the efficacy of green tea is likely to be mediated by the effects of EGCG, whilst the main bioactive constituents of black tea are the theaflavins. It is clear that EGCG has been the polyphenolic compound of choice most extensively investigated both *in vivo* and *in vitro*, and therefore future studies should now address the effects of other important tea polyphenols, especially theaflavins. While many of the mechanisms underpinning their beneficial effects have been highlighted, it has become clear that apart from the classical anti-oxidant properties, tea polyphenols may in part incur neuroprotection in PD through specific cell signalling pathways and prevention of αS aggregation.

Finally, the extent of their contribution *in vivo*, and at physiological relevant concentrations remains to be ascertained. Conflicting epidemiological inferences and discrepancies between *in vitro* and *in vivo* studies may be due to erratic bioavailability of tea polyphenols. Although more urgent work needs to be done to prove whether tea polyphenols can be translated in PD patients and to clarify their absorption, metabolism, and potential toxicity in humans, their multiple biological activities, and especially in combination with other compounds that possess neuroprotective moieties, may offer a superior therapeutic effect in delaying the initiation and progression of PD.

References

Abd El Mohsen MM, Kuhnle G, Rechner AR, Schroeter H, Rose S, Jenner P, Rice-Evans CA (2002) Uptake and metabolism of epicatechin and its access to the brain after oral ingestion. Free Radic Biol Med 33(12):1693–1702

Adachi N, Tomonaga S, Tachibana T, Denbow DM, Furuse M (2006) (−)-Epigallocatechin gallate attenuates acute stress responses through GABAergic system in the brain. Eur J Pharmacol 531(1–3):171–175

Aherne SA, O'Brien NM (2002) Dietary flavonols: chemistry, food content, and metabolism. Nutrition 18(1):75–81

Albani D, Polito L, Signorini A, Forloni G (2010) Neuroprotective properties of resveratrol in different neurodegenerative disorders. Biofactors 36(5):370–376

Amit T, Avramovich-Tirosh Y, Youdim MBH, Mandel S (2008) Targeting multiple Alzheimer's disease etiologies with multimodal neuroprotective and neurorestorative iron chelators. FASEB J 22(5):1296–1305

Anandhan A, Janakiraman U, Manivasagam T (2012a) Theaflavin ameliorates behavioral deficits, biochemical indices and monoamine transporters expression against subacute 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mouse model of Parkinson's disease. Neuroscience 218:257–267

Anandhan A, Tamilselvam K, Radhiga T, Rao S, Essa MM, Manivasagam T (2012b) Theaflavin, a black tea polyphenol, protects nigral dopaminergic nerons against chronic MPTP/probenecid induced Parkinson's disease. Brain Res 1433:104–113

Aron PM, Kennedy JA (2008) Flavan-3-ols: nature, occurrence and biological activity. Mol Nutr Food Res 52(1):79–104

Ascherio A, Zhang SM, Hernan MA, Kawachi I, Colditz GA, Speizer FE, Willett WC (2001) Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. Ann Neurol 50:56–63

Astill C, Birch MR, Dacombe C, Humphrey PG, Martin PT (2001) Factors affecting the caffeine and polyphenol contents of black and green tea infusions. J Agric Food Chem 49(11):5340–5347

Auger C, Mullen W, Hara Y, Crozier A (2008) Bioavailability of polyphenon E flavan-3-ols in humans with an ileostomy. J Nutr 138(8):1542

Bae SY, Kim S, Hwang H, Kim HK, Yoon HC, Kim JH, Lee S, Kim TD (2010) Amyloid formation and disaggregation of α-synuclein and its tandem repeat (α-TR). Biochem Biophys Res Commun 400:531–536

Balentine DA, Wiseman SA, Bouwens LC (1997) The chemistry of tea flavonoids. Crit Rev Food Sci Nutr 37:693–704

Barnham KJ, Bush AI (2008) Metals in Alzheimer's and Parkinson's diseases. Curr Opin Chem Biol 12(2):222–228

Bastianetto S (2002) Red wine consumption and brain aging. Nutrition 18(5):432–433

Bastianetto S, Quirion R (2004) Natural antioxidants and neurodegenerative diseases. Front Biosci 9:3447–3452

Bastianetto S, Yao Z-X, Papadopoulos V, Quirion R (2006) Neuroprotective effects of green and black teas and their catechin gallate esters against beta-amyloid-induced toxicity. Eur J Neurosci 23(1):55–64

Beecher GR (2003) Overview of dietary flavonoids: nomenclature, occurrence and intake. J Nutr 133(10):3254

Berhanu WM, Masunov AE (2010) Natural polyphenols as inhibitors of amyloid aggregation. Molecular dynamics study of GNNQQNY heptapeptide decamer. Biophys Chem 149(1–2):12–21

Bieschke J, Russ J, Friedrich RP, Ehrnhoefer DE, Wobst H, Neugebauer K, Wanker EE (2010) EGCG remodels mature alpha-synuclein and amyloid-beta fibrils and reduces cellular toxicity. Proc Natl Acad Sci U S A 107:7710–7715

Blazovics A, Lugasi A, Kemeny T, Hagymasi K, Kery A (2000) Membrane stabilising effects of natural polyphenols and flavonoids from Sempervivum tectorum on hepatic microsomal mixed-function oxidase system in hyperlipidemic rats. J Ethnopharmacol 73(3):479–485

Braak H, Ghebremedhin E, Rub U, Bratzke H, Del Tredici K (2004) Stages in the development of Parkinson's disease-related pathology. Cell Tissue Res 318(1):121–134

Büeler H (2009) Impaired mitochondrial dynamics and function in the pathogenesis of Parkinson's disease. Exp Neurol 218:235–246

Calixto JB, Campos MM, Otuki MF, Santos AR (2004) Anti-inflammatory compounds of plant origin. Part II. Modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. Planta Med 70(2):93–103

Camilleri A, Vassallo N (2014) The centrality of mitochondria in the pathogenesis and treatment of Parkinson's disease. CNS Neurosci Ther 20(7):591–602

Camilleri A, Zarb C, Caruana M, Ostermeier U, Ghio S, Hogen T, Schmidt F, Giese A, Vassallo N (2013) Mitochondrial membrane permeabilisation by amyloid aggregates and protection by polyphenols. Biochim Biophys Acta 1828(11):2532–2543

Campos-Esparza R, Torres-Ramos MA (2010) Neuroprotection by natural polyphenols: molecular mechanisms. Cent Nerv Syst Agents Med Chem 10:269–277

Cao G, Giovanoni M, Prior RL (1996) Antioxidant capacity in different tissues of young and old rats. Proc Soc Exp Biol Med 211(4):359–365

Caruana M, Vassallo N (2011) The potential role of dietary polyphenols in Parkinson's disease. Malta Med J 23(3):52–55

Caruana M, Vassallo N (2014) Select polyphenols that protect mitochondria against amyloid aggregates in Alzheimer's and Parkinson's disease. Xjenza Online 2:17–25

Caruana M, Hogen T, Levin J, Hillmer A, Giese A, Vassallo N (2011) Inhibition and disaggregation of α-synuclein oligomers by natural polyphenolic compounds. FEBS Lett 585(8):1113–1120

Caruana M, Neuner J, Högen T, Schmidt F, Scerri C, Giese A, Vassallo N (2012) Polyphenolic compounds are novel protective agents against lipid membrane damage by α-synuclein aggregates in vitro. Biochim Biophys Acta 1818(11):2502–2510

Chan DK, Woo J, Ho SC, Pang CP, Law LK, Ng PW, Hung WT, Kwok T, Hui E, Orr K, Leung MF, Kay R (1998) Genetic and environmental risk factors for Parkinson's disease in a Chinese population. J Neurol Neurosurg Psychiatry 65(5):781–784

Chan P, Qin Z, Zheng Z, Zhang L, Fang X, Sun F, Gu Z, Chen S, Ma J, Meng C, Langston JW, Tanner CM (2009) A randomized, double-blind, placebo controlled, delayed start study to assess safety, tolerability and efficacy of green tea polyphenols in Parkinson's disease. XVIII WFN World Congress on Parkinson's Disease and Related Disorders, Miami Beach, 13–16 Dec 2009

Chaturvedi RK, Shukla S, Seth K, Chauhan S, Sinha C, Shukla Y, Agrawal AK (2006) Neuroprotective and neurorescue effect of black tea extract in 6-hydroxydopamine-lesioned rat model of Parkinson's disease. Neurobiol Dis 22(2):421–434

Checkoway H, Powers K, Smith-Weller T, Franklin GM, Longstreth WT, Swanson PD (2002) Parkinson's disease risks associated with cigarette smoking, alcohol consumption, and caffeine intake. Am J Epidemiol 155(8):732–738

Chen CM, Lin JK, Liu SH, Lin-Shiau SY (2008) Novel regimen through combination of memantine and tea polyphenol for neuroprotection against brain excitotoxicity. J Neurosci Res 86(12):2696–2704

Chen R, Wang JB, Zhang XQ, Ren J, Zeng CM (2011) Green tea polyphenol epigallocatechin-3-gallate (EGCG) induced intermolecular cross-linking of membrane proteins. Arch Biochem Biophys 507(2):343–349

Chen M, Wang T, Yue F, Li X, Wang P, Li Y, Chan P, Yu S (2014) Tea polyphenols alleviate motor impairments, dopaminergic neuronal injury, and cerebral α-synuclein aggregation in MPTP-intoxicated parkinsonian monkeys. Neuroscience 286:383–392

Choi JY, Park CS, Kim DJ, Cho MH, Jin BK, Pie JE, Chung WG (2002) Prevention of nitric oxide-mediated 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's disease in mice by tea phenolic epigallocatechin 3-gallate. Neurotoxicology 23(3):367–374

Choi YJ, Jeong YJ, Lee YJ, Kwon HM, Kang YH (2005) (−)Epigallocatechin gallate and quercetin enhance survival signaling in response to oxidant-induced human endothelial apoptosis. J Nutr 135(4):707–713

Choi DY, Lee YJ, Hong JT, Lee HJ (2012) Antioxidant properties of natural polyphenols and their therapeutic potentials for Alzheimer's disease. Brain Res Bull 87(2–3):144–153

Chow HH, Cai Y, Hakim IA, Crowell JA, Shahi F, Brooks CA, Dorr RT, Hara Y, Alberts DS (2003) Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. Clin Cancer Res 9(9):3312–3319

Chow HH, Hakim IA, Vining DR, Crowell JA, Ranger-Moore J, Chew WM, Celaya CA, Rodney SR, Hara Y, Alberts DS (2005) Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of Polyphenon E in healthy individuals. Clin Cancer Res 11(12):4627–4633

Chun OK, Chung SJ, Song WO (2007) Estimated dietary flavonoid intake and major food sources of U.S. adults. J Nutr 137(5):1244–1252

Conway KA, Rochet JC, Bieganski RM, Lansbury PT (2001) Kinetic stabilization of the alpha-synuclein protofibril by a dopamine-alpha-synuclein adduct. Science 294(5545):1346–1349

Costa J, Lunet N, Santos C, Santos J, Vaz-Carneiro A (2010) Caffeine exposure and the risk of Parkinson's disease: a systematic review and meta-analysis of observational studies. J Alzheimers Dis 20:221–238

Crozier A, Jaganath IB, Clifford MN (2009) Dietary phenolics: chemistry, bioavailability and effects on health. Nat Prod Rep 26(8):1001–1043

D'Archivio M, Filesi C, Di Benedetto R, Gargiulo R, Giovannini C, Masella R (2007) Polyphenols, dietary sources and bioavailability. Ann Ist Super Sanita 43:348–361

Dajas F, Rivera F, Blasina F, Arredondo F, Echeverry C, Lafon L, Morquio A, Heinzen H, Heizen H (2003) Cell culture protection and in vivo neuroprotective capacity of flavonoids. Neurotox Res 5(6):425–432

de Lau LM, Breteler MM (2006) Epidemiology of Parkinson's disease. Lancet Neurol 5:525–535

Dorsey ER, Constantinescu R, Thompson JP, Biglan KM, Holloway RG, Kieburtz K, Marshall FJ, Ravina BM, Schifitto G, Siderowf A, Tanner CM (2007) Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. Neurology 68:384–386

Duchnowicz P, Bors M, Podsędek A, Koter-Michalak M, Broncel M (2012) Effect of polyphenols extracts from Brassica vegetables on erythrocyte membranes (in vitro study). Environ Toxicol Pharmacol 34(3):783–790

Ehrnhoefer DE, Bieschke J, Boeddrich A, Herbst M, Masino L, Lurz R, Engemann S, Pastore A, Wanker EE (2008) EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers. Nat Struct Mol Biol 15:558–566

Engel MFM, van den Akker CC, Schleeger M, Velikov KP, Koenderink GH, Bonn M (2012) The polyphenol EGCG inhibits amyloid formation less efficiently at phospholipid interfaces than in bulk solution. J Am Chem Soc 134(36):14781–14788

Erdman JW, Balentine D, Arab L, Beecher G, Dwyer JT, Folts J, Harnly J, Hollman P, Keen CL, Mazza G, Messina M, Scalbert A, Vita J, Williamson G, Burrowes J (2007) Flavonoids and heart health: proceedings of the ILSI North America Flavonoids Workshop, May 31-June 1, 2005, Washington, DC. J Nutr 137(3 Suppl 1):737

Fall PA, Fredrikson M, Axelson O, Granérus AK (1999) Nutritional and occupational factors influencing the risk of Parkinson's disease: a case–control study in south eastern Sweden. Mov Disord 14:28–37

Faria A, Pestana D, Teixeira D, Couraud PO, Romero I, Weksler B, de Freitas V, Mateus N, Calhau C (2011) Insights into the putative catechin and epicatechin transport across blood–brain barrier. Food Funct 2(1):39–44

Frei B, Higdon JV (2003) Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. J Nutr 133:3275–3284

Friedman A, Galazka-Friedman J (2001) The current state of free radicals in Parkinson's disease: nigral iron as a trigger of oxidative stress. Adv Neurol 86:137–142

Fukae J, Mizuno Y, Hattori N (2007) Mitochondrial dysfunction in Parkinson's disease. Mitochondrion 7(1–2):58–62

Gao X, Cassidy A, Schwarzschild MA, Rimm EB, Ascherio A (2012) Habitual intake of dietary flavonoids and risk of Parkinson disease. Neurology 78(15):1138–1145

Gazit E (2002) A possible role for π-stacking in the self-assembly of amyloid fibrils. FASEB J 16:77–83

Gerlach M, Maetzler W, Broich K, Hampel H, Rems L, Reum T, Riederer P, Stoffler A, Streffer J, Berg D (2012) Biomarker candidates of neurodegeneration in Parkinson's disease for the evaluation of disease-modifying therapeutics. J Neural Transm 119(1):39–52

Ghosh D, Scheepens A (2009) Vascular action of polyphenols. Mol Nutr Food Res 53(3):322–331

Graham HN (1992) Green tea composition, consumption, and polyphenol chemistry. Prev Med 21(3):334–350

Grelle G, Otto A, Lorenz M, Frank RF, Wanker EE, Bieschke J (2011) Black tea theaflavins inhibit formation of toxic amyloid-β and α-synuclein fibrils. Biochemistry 50(49):10624–10636

Guo S, Yan J, Yang T, Yang X, Bezard E, Zhao B (2007) Protective effects of green tea polyphenols in the 6-OHDA rat model of Parkinson's disease through inhibition of ROS-NO pathway. Biol Psychiatry 62(12):1353–1362

Haenen GR, Paquay JB, Korthouwer RE, Bast A (1997) Peroxynitrite scavenging by flavonoids. Biochem Biophys Res Commun 236(3):591–593

Hancock DB, Martin ER, Stajich JM, Jewett R, Stacy MA, Scott BL, Vance JM, Scott WK (2007) Smoking, caffeine, and nonsteroidal anti-inflammatory drugs in families with Parkinson disease. Arch Neurol 64:576–580

Harbowy ME, Ballentine DA (1997) Tea chemistry. Crit Rev Plant Sci 16:415–480

Hartmann A, Hirsch EC (2001) Parkinson's disease. The apoptosis hypothesis revisited. Adv Neurol 86:143–153

Hendrich AB (2006) Flavonoid-membrane interactions: possible consequences for biological effects of some polyphenolic compounds. Acta Pharmacol Sin 27:27–40

Henning SM, Choo JJ, Heber D (2008) Nongallated compared with gallated flavan-3-ols in green and black tea are more bioavailable. J Nutr 138(8):1534

Hernan MA, Takkouche B, Caamano-Isorna F, Gestal-Otero JJ (2002) A meta-analysis of coffee drinking, cigarette smoking, and the risk of Parkinson's disease. Ann Neurol 52(3):276–284

Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D (1993) Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 342(8878):1007–1011

Higdon JV, Frei B (2003) Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. Crit Rev Food Sci Nutr 43(1):89–143

Hollman PC, Van het Hof KH, Tijburg LB, Katan MB (2001) Addition of milk does not affect the absorption of flavonols from tea in man. Free Radic Res 34:297–300

Hu G, Bidel S, Jousilahti P, Antikainen R, Tuomilehto J (2007) Coffee and tea consumption and the risk of Parkinson's disease. Mov Disord 22(15):2242–2248

Huang CC, Wu WB, Fang JY, Chiang HS, Chen SK, Chen BH, Chen YT, Hung CF (2007) (−)-Epicatechin-3-gallate, a green tea polyphenol is a potent agent against UVB-induced damage in HaCaT keratinocytes. Molecules 12(8):1845–1858

Hudson SA, Ecroyd H, Dehle FC, Musgrave IF, Carver JA (2009) (−)-epigallocatechin-3-gallate (EGCG) maintains kappa-casein in its pre-fibrillar state without redirecting its aggregation pathway. J Mol Biol 392(3):689–700

Hwang SL, Yen GC (2009) Modulation of Akt, JNK, and p38 activation is involved in citrus flavonoid-mediated cytoprotection of PC12 cells challenged by hydrogen peroxide. J Agric Food Chem 57:2576–2582

Jankovic J (2008) Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry 79(4):368–376

Jeong JH, Kim HJ, Lee TJ, Kim MK, Park ES, Choi BS (2004) Epigallocatechin 3-gallate attenuates neuronal damage induced by 3-hydroxykynurenine. Toxicology 195(1):53–60

Jimenez-Del-Rio M, Guzman-Martinez C, Velez-Pardo C (2010) The effects of polyphenols on survival and locomotor activity in *Drosophila melanogaster* exposed to iron and paraquat. Neurochem Res 35:227–238

Johnston TH, Brotchie JM (2004) Drugs in development for Parkinson's disease. Curr Opin Investig Drugs 5:720–726

Kandinov B, Giladi N, Korczyn AD (2009) Smoking and tea consumption delay onset of Parkinson's disease. Parkinsonism Relat Disord 15(1):41–46

Kaur D, Yantiri F, Rajagopalan S, Kumar J, Mo JQ, Boonplueang R, Viswanath V, Jacobs R, Yang L, Beal MF, DiMonte D, Volitaskis I, Ellerby L, Cherny RA, Bush AI, Andersen JK (2003) Genetic or pharmacological iron chelation prevents MPTP-induced neurotoxicity in vivo: a novel therapy for Parkinson's disease. Neuron 37(6):899–909

Kazantsev AG, Kolchinsky AM (2008) Central role of alpha-synuclein oligomers in neurodegeneration in Parkinson disease. Arch Neurol 65(12):1577–1581

Khokhar S, Magnusdottir SGM (2002) Total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom. J Agric Food Chem 50(3):565–570

Kishido T, Unno K, Yoshida H, Choba D, Fukutomi R, Asahina S, Iguchi K, Oku N, Hoshino M (2007) Decline in glutathione peroxidase activity is a reason for brain senescence: consumption of green tea catechin prevents the decline in its activity and protein oxidative damage in ageing mouse brain. Biogerontology 8(4):423–430

Kumamoto M, Sonda T, Nagayama K, Tabata M (2001) Effects of pH and metal ions on antioxidative activities of catechins. Biosci Biotechnol Biochem 65(1): 126–132

Kumar S, Pandey AK (2013) Chemistry and biological activities of flavonoids: an overview. Scientific World Journal 2013:162750

Kyle JA, Morrice PC, McNeill G, Duthie GG (2007) Effects of infusion time and addition of milk on content and absorption of polyphenols from black tea. J Agric Food Chem 55(12):4889–4894

Kyoung AK, Zhi HW, Rui Z, Mei JP, Ki CK, Sam SK, Young WK, Jongsung L, Deokhoon P, Jin WH (2010) Myricetin protects cells against oxidative stress-induced apoptosis via regulation of PI3K/Akt and MAPK signalling pathways. Int J Mol Sci 11:4348–4360

Lashuel HA, Petre BM, Wall J, Simon M, Nowak RJ, Walz T, Lansbury PT Jr (2002) Alpha-synuclein, especially the Parkinson's disease-associated mutants, forms pore-like annular and tubular protofibrils. J Mol Biol 322:1089–1102

Leaver KR, Allbutt HN, Creber NJ, Kassiou M, Henderson JM (2009) Oral pre-treatment with epigallocatechin gallate in 6-OHDA lesioned rats produces subtle symptomatic relief but not neuroprotection. Brain Res Bull 80(6):397–402

Lee MJ, Maliakal P, Chen L, Meng X, Bondoc FY, Prabhu S, Lambert G, Mohr S, Yang CS (2002) Pharmacokinetics of tea catechins after ingestion of green tea and (−)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. Cancer Epidemiol Biomark Prev 11:1025–1032

Leung LK, Su Y, Chen R, Zhang Z, Huang Y, Chen ZY (2001) Theaflavins in black tea and catechins in green tea are equally effective antioxidants. J Nutr 131(9):2248–2251

Levites Y, Weinreb O, Maor G, Youdim MB, Mandel S (2001) Green tea polyphenol (−)-epigallocatechin-3-gallate prevents N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dopaminergic neurodegeneration. J Neurochem 78:1073–1082

Levites Y, Youdim MB, Maor G, Mandel S (2002a) Attenuation of 6-hydroxydopamine (6-OHDA)-induced nuclear factor-kappaB (NF-kappaB) activation and celldeath by tea extracts in neuronal cultures. Biochem Pharmacol 63(1):21–29

Levites Y, Amit T, Youdim MB, Mandel S (2002b) Involvement of protein kinase C activation and cell survival/ cell cycle genes in green tea polyphenol (−)-epigallocatechin 3-gallate neuroprotective action. J Biol Chem 277:30574–30580

Li S, Schoenberg BS, Wang CC, Cheng X-M, Rui D-Y, Bolis CL, Schoenberg BG (1985) A prevalence survey of Parkinson's disease and other movement disorders in the People's Republic of China. Arch Neurol 42:655–657

Li FJ, Ji HF, Shen L (2012) A meta-analysis of tea drinking and risk of Parkinson's disease. Scientific World Journal 2012:923464

Logroscino G (2005) The role of early life environmental risk factors in Parkinson disease: what is the evidence? Environ Health Perspect 113:1234–1238

López-Lázaro M (2009) Distribution and biological activities of the flavonoid luteolin. Mini Rev Med Chem 9:31–59

Lotito SB, Frei B (2006) Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? Free Radic Biol Med 41(12):1727–1746

Luczaj W, Skrzydlewska E (2005) Antioxidative properties of black tea. Prev Med 40:910–918

Mandel S, Youdim MB (2004) Catechin polyphenols: neurodegeneration and neuroprotection in neurodegenerative diseases. Free Radic Biol Med 37:304–317

Mandel S, Weinreb O, Amit T, Youdim MB (2004) Cell signaling pathways in the neuroprotective actions of the green tea polyphenol (−)-epigallocatechin-3-gallate: Implications for neurodegenerative diseases. J Neurochem 88:1555–1569

Mandel SA, Amit T, Weinreb O, Reznichenko L, Youdim MBH (2008) Simultaneous manipulation of multiple brain targets by green tea catechins: a potential neuroprotective strategy for Alzheimer and Parkinson diseases. CNS Neurosci Ther 14(4):352–365

Masuda M, Suzuki N, Taniguchi S, Oikawa T, Nonaka T, Iwatsubo T, Hisanaga S-i, Goedert M, Hasegawa M (2006) Small molecule inhibitors of alpha-synuclein filament assembly. Biochemistry 45(19):6085–6094

McGeer PL, Yasojima K, McGeer EG (2001) Inflammation in Parkinson's disease. Adv Neurol 86:83–89

McKay DL, Blumberg JB (2002) The role of tea in human health: an update. JACN 21:1–13

Meng X, Munishkina LA, Fink AL, Uversky VN (2009) Molecular mechanisms underlying the flavonoid-induced inhibition of alpha-synuclein fibrillation. Biochemistry 48(34):8206–8224

Meng X, Munishkina LA, Fink AL, Uversky VN (2010) Effects of various flavonoids on the α-synuclein fibrillation process. Parkinsons Dis 2010:650794

Mercer LD, Kelly BL, Horne MK, Beart PM (2005) Dietary polyphenols protect dopamine neurons from oxidative insults and apoptosis: investigations in primary rat mesencephalic cultures. Biochem Pharmacol 69(2):339–345

Moon JK, Shibamoto T (2009) Antioxidant assays for plant and food components. J Agric Food Chem 57(5):1655–1666

Na HK, Kim EH, Jung JH, Lee HH, Hyun JW, Surh YJ (2008) (−)-Epigallocatechin gallate induces Nrf2-mediated antioxidant enzyme expression via activation of PI3K and ERK in human mammary epithelial cells. Arch Biochem Biophys 476(2):171–177

Nakagawa K, Miyazawa T (1997) Absorption and distribution of tea catechin, (−)-epigallocatechin-3-gallate, in the rat. J Nutr Sci Vitaminol (Tokyo) 43(6):679–684

Nakagawa T, Yokozawa T (2002) Direct scavenging of nitric oxide and superoxide by green tea. Food Chem Toxicol 40(12):1745–1750

Nanjo F, Goto K, Seto R, Suzuki M, Sakai M, Hara Y (1996) Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical. Free Radic Biol Med 21:895–902

Obrenovich ME, Nair NG, Beyaz A, Aliev G, Reddy VP (2010) The role of polyphenolic antioxidants in health, disease, and aging. Rejuvenation Res 13(6):631–643

Oku N, Matsukawa M, Yamakawa S, Asai T, Yahara S, Hashimoto F, Akizawa T (2003) Inhibitory effect of green tea polyphenols on membrane-type 1 matrix metalloproteinase, MT1-MMP. Biol Pharm Bull 26(9):1235–1238

Olanow CW, Rascol O, Hauser R, Feigin PD, Jankovic J, Lang A, Langston W, Melamed E, Poewe W, Stocchi F, Tolosa E, ADAGIO Study Investigators (2009) A double-blind, delayed-start trial of rasagiline in Parkinson's disease. N Engl J Med 361(13):1268–1278

Ono K, Yamada M (2006) Antioxidant compounds have potent anti-fibrillogenic and fibril-destabilizing effects for alpha-synuclein fibrils *in vitro*. J Neurochem 97:105–115

Paganini-Hill A (2001) Risk factors for Parkinson's disease: the Leisure World cohort study. Neuroepidemiology 20:118–124

Pan T, Jankovic J, Le W (2003) Potential therapeutic properties of green tea polyphenols in Parkinson's disease. Drugs Aging 20(10):711–721

Pan T, Fei J, Zhou X, Jankovic J, Le W (2004) Effects of green tea polyphenols on dopamine uptake and on MPPS-induced dopamine neuron injury. Life Sci 72:1073–1083

Periquet M, Fulga T, Myllykangas L, Schlossmacher MG, Feany MB (2007) Aggregated alpha-synuclein mediates dopaminergic neurotoxicity in vivo. J Neurosci 27(12):3338–3346

Perron NR, Brumaghim JL (2009) A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. Cell Biochem Biophys 53(2):75–7100

Perron NR, Hodges JN, Jenkins M, Brumaghim JL (2008) Predicting how polyphenol antioxidants prevent DNA damage by binding to iron. Inorg Chem 47(14):6153–6161

Perron NR, Wang HC, Deguire SN, Jenkins M, Lawson M, Brumaghim JL (2010) Kinetics of iron oxidation upon polyphenol binding. Dalton Trans 39(41):9982–9987

Porat Y, Mazor Y, Efrat S, Gazit E (2004) Inhibition of islet amyloid polypeptide fibril formation: a potential role for heteroaromatic interactions. Biochemistry 43(45):14454–14462

Porat Y, Abramowitz A, Gazit E (2006) Inhibition of amyloid fibril formation by polyphenols: structural similarity and aromatic interactions as a common inhibition mechanism. Chem Biol Drug Des 67(1):27–37

Preux PM, Condet A, Anglade C, Druet-Cabanac M, Debrock C, Macharia W, Couratier P, Boutros-Toni F, Dumas M (2000) Parkinson's disease and environmental factors. Matched caseecontrol study in the Limousin region, France. Neuroepidemiology 19(6):333–337

Quintana BJ, Allam MF, Del Castillo AS, Navajas RF (2009) Parkinson's disease and tea: a quantitative review. J Am Coll Nutr 28(1):1–6

Quist A, Doudevski I, Lin H, Azimova R, Ng D, Frangione B, Kagan B, Ghiso J, Lal R (2005) Amyloid ion channels: a common structural link for protein-misfolding disease. Proc Natl Acad Sci U S A 102(30):10427–10432

Rahman M, Riaz M, Desai UR (2007) Synthesis of biologically relevant biflavanoids; a review. Chem Biodivers 4:2495–2527

Ramassamy C (2006) Emerging role of polyphenolic compounds in the treatment of neurodegenerative diseases: a review of their intracellular targets. Eur J Pharmacol 545:51–64

Ramsden DB, Parsons RB, Ho SL, Waring RH (2001) The aetiology of idiopathic Parkinson's disease. Mol Pathol 54(6):369–380

Reznichenko L, Amit T, Youdim MBH, Mandel S (2005) Green tea polyphenol (−)-epigallocatechin-3-gallate induces neurorescue of long-term serum-deprived PC12 cells and promotes neurite outgrowth. J Neurochem 93(5):1157–1167

Reznichenko L, Kalfon L, Amit T, Youdim MBH, Mandel SA (2010) Low dosage of rasagiline and epigallocatechin gallate synergistically restored the nigrostriatal axis in MPTP-induced parkinsonism. Neurodegener Dis 7(4):219–231

Rietveld A, Wiseman S (2003) Antioxidant effects of tea: evidence from human clinical trials. J Nutr 133:3285–3292

Ritz B, Ascherio A, Checkoway H, Marder KS, Nelson LM, Rocca WA, Ross GW, Strickland D, Van Den Eeden SK, Gorell J (2007) Pooled analysis of tobacco use and risk of Parkinson disease. Arch Neurol 64(7):990–997

Roowi S, Stalmach A, Mullen W, Lean ME, Edwards CA, Crozier A (2010) Green tea flavan-3-ols: colonic degradation and urinary excretion of catabolites by humans. J Agric Food Chem 58:1296–1304

Saaksjarvi K, Knekt P, Rissanen H, Laaksonen MA, Reunanen A, Mannisto S (2008) Prospective study of coffee consumption and risk of Parkinson's disease. Eur J Clin Nutr 62:908–915

Saura-Calixto F, Serrano J, Goñi I (2007) Intake and bioaccessibility of total polyphenols in a whole diet. Food Chem 101:492–501

Scalbert A, Williamson G (2000) Dietary intake and bioavailability of polyphenols. J Nutr 130(8S Suppl):85

Scalbert A, Manach C, Morand C, Remesy C, Jimenez L (2005) Dietary polyphenols and the prevention of diseases. Crit Rev Food Sci Nutr 45(4):287–306

Schaffer S, Halliwell B (2012) Do polyphenols enter the brain and does it matter? Some theoretical and practical considerations. Genes Nutr 7(2):99–109

Schrag A, Ben-Shlomo Y, Quinn N (2002) How valid is the clinical diagnosis of Parkinson's disease in the community? J Neurol Neurosurg Psychiatry 73:529–534

Schroeter H, Bahia P, Spencer JPE, Sheppard O, Rattray M, Cadenas E, Rice-Evans C, Williams RJ (2007) (−)Epicatechin stimulates ERK-dependent cyclic AMP response element activity and up-regulates GluR2 in cortical neurons. J Neurochem 101(6):1596–1606

Selkoe DJ (2008) Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior. Behav Brain Res 192(1):106–113

Sharma M, Dhamgaye S, Singh A, Prasad R (2012) Lipidome analysis reveals antifungal polyphenol curcumin affects membrane lipid homeostasis. Front Biosci 4:1195–1209

Shults CW (2006) Lewy bodies. Proc Natl Acad Sci U S A 103:1661–1668

Sirk TW, Brown EF, Sum AK, Friedman M (2008) Molecular dynamics study on the biophysical interactions of seven green tea catechins with lipid bilayers of cell membranes. J Agric Food Chem 56:7750–7758

Sirk TW, Friedman M, Brown EF (2011) Molecular binding of black tea theaflavins to biological membranes: relationship to bioactivities. J Agric Food Chem 59(8):3780–3787

Skovronsky DM, Lee VM, Trojanowski JQ (2006) Neurodegenerative diseases: new concepts of pathogenesis and their therapeutic implications. Annu Rev Pathol Mech Dis 1:151–170

Song WO, Chun OK (2008) Tea is the major source of flavan-3-ol and flavonol in the U.S. diet. J Nutr 138(8):1547

Soto C (2003) Unfolding the role of protein misfolding in neurodegenerative diseases. Nat Rev Neurosci 4:49–60

Soto-Otero R, Mendez-Alvarez E, Hermida-Ameijeiras A, Munoz Patino AM, Labaneira-Garcıa JL (2000) Autoxidation and neurotoxicity of 6-hydrodoe in the presence of some antioxidants: potential implication in relation to the pathogenesis of Parkinson's disease. J Neurochem 74:1605–1612

Spencer JPE (2008) Flavonoids: modulators of brain function? Br J Nutr 99:60–77

Spencer JPE, Vauzour D, Rendeiro C (2009) Flavonoids and cognition: the molecular mechanisms underlying their behavioural effects. Arch Biochem Biophys 492(1–2):1–9

Stalmach A, Troufflard S, Serafini M, Crozier A (2009) Absorption, metabolism and excretion of Choladi green tea flavan-3-ols by humans. Mol Nutr Food Res 53(Suppl 1):44–53

Stevenson DE, Hurst RD (2007) Polyphenolic phytochemicals – just antioxidants or much more? Cell Mol Life Sci 64:2900–2916

Sumpio BE, Cordova AC, Berke-Schlessel DW, Qin F, Chen QH (2006) Green tea, the "Asian paradox," and cardiovascular disease. J Am Coll Surg 202(5):813–825

Tan EK, Tan C, Fook-Chong SMC, Lum SY, Chai A, Chung H, Shen H, Zhao Y, Teoh ML, Yih Y, Pavanni R, Chandran VR, Wong MC (2003) Dose-dependent protective effect of coffee, tea, and smoking in Parkinson's disease: a study in ethnic Chinese. J Neurol Sci 216(1):163–167

Tan LC, Koh W-P, Yuan J-M, Wang R, Au W-L, Tan JH, Tan E-K, Yu MC (2008) Differential effects of black versus green tea on risk of Parkinson's disease in the Singapore Chinese Health Study. Am J Epidemiol 167(5):553–560

Tanner CM, Ottman R, Goldman SM, Ellenberg J, Chan P, Mayeux R, Langston JW (1999) Parkinson disease in twins: an etiologic study. JAMA 281:341–346

Tanner CM, Goldman SM, Aston DA, Ottman R, Ellenberg J, Mayeux R, Langston JW (2002) Smoking and Parkinson's disease in twins. Neurology 58(4):581–588

Toda T (2007) Molecular genetics of Parkinson's disease. Brain Nerve 59(8):815–823

Tsao R (2010) Chemistry and biochemistry of dietary polyphenols. Nutrients 2(12):1231–1246

Vafeiadou K, Vauzour D, Spencer JPE (2007) Neuroinflammation and its modulation by flavonoids. Endocr Metab Immune Disord Drug Targets 7(3): 211–224

Vassallo N (ed) (2008) Polyphenols and health: new and recent advantages. Nova, New York

Vauzour D (2012) Dietary polyphenols as modulators of brain functions: biological actions and molecular mechanisms underpinning their beneficial effects. Oxid Med Cell Longev 2012:914273

Vauzour D, Vafeiadou K, Rice-Evans C, Williams RJ, Spencer JP (2007) Activation of pro-survival Akt and ERK1/2 signalling pathways underlie the anti-apoptotic effects of flavanones in cortical neurons. J Neurochem 103(4):1355–1367

Vauzour D, Ravaioli G, Vafeiadou K, Rodriguez-Mateos A, Angeloni C, Spencer JPE (2008) Peroxynitrite induced formation of the neurotoxins 5-S-cysteinyl-dopamine and DHBT-1: implications for Parkinson's disease and protection by polyphenols. Arch Biochem Biophys 476(2):145–151

Verstraeten SV, Keen CL, Schmitz HH, Fraga CG, Oteiza PI (2003) Flavan-3-ols and procyanidins protect liposomes against lipid oxidation and disruption of the bilayer structure. Free Radic Biol Med 34(1):84–92

Wang H, Helliwell K (2001) Determination of flavonols in green and black tea leaves and green tea infusions by high performance liquid chromatography. Food Res Int 34(2–3):223–227

Wang SH, Liu FF, Dong XY, Sun Y (2010) Thermodynamic analysis of the molecular interactions between amyloid beta-peptide 42 and (−)-epigallocatechin-3-gallate. J Phys Chem B 114(35): 11576–11583

Weinreb O, Mandel S, Amit T, Youdim MB (2004) Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases. J Nutr Biochem 15:506–516

Weinreb O, Amit T, Youdim MB (2008) The application of proteomics for studying the neurorescue activity of the polyphenol (−)-epigallocatechin-3-gallate. Arch Biochem Biophys 476:152–160

Weinreb O, Amit T, Mandel S, Kupershmidt L, Youdim MB (2010) Neuroprotective multifunctional iron chelators: from redox-sensitive process to novel therapeutic opportunities. Antioxid Redox Signal 13:919–949

Wheeler DS, Wheeler WJ (2004) The medicinal chemistry of tea. Drug Dev Res 61:45–65

Williams RJ, Spencer JPE, Rice-Evans C (2004) Flavonoids: antioxidants or signalling molecules? Free Radic Biol Med 36(7):838–849

Williamson G, Manach C (2005) Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. Am J Clin Nutr 81(Suppl 1):243–255

Winner B, Jappelli R, Maji SK, Desplats PA, Boyer L, Aigner S, Hetzer C, Loher T, Vilar M, Campioni S, Tzitzilonis C, Soragni A, Jessberger S, Mira H, Consiglio A, Pham E, Masliah E, Gage FH, Riek R (2011) *In vivo* demonstration that alpha-synuclein oligomers are toxic. Proc Natl Acad Sci U S A 108: 4194–4199

Wirdefeldt K, Gatz M, Schalling M, Pedersen NL (2004) No evidence for heritability of Parkinson disease in Swedish twins. Neurology 63(2):305–311

Wirdefeldt K, Adami H-O, Cole P, Trichopoulos D, Mandel J (2011) Epidemiology and etiology of Parkinson's disease: a review of the evidence. Eur J Epidemiol 26(Suppl 1):1–58

Wu AH, Arakawa K, Stanczyk FZ, Van Den Berg D, Koh WP, Yu MC (2005) Tea and circulating estrogen levels in postmenopausal Chinese women in Singapore. Carcinogenesis 26(5):976–980

Xi YD, Yu HL, Ding J, Ma WW, Yuan LH, Feng JF, Xiao YX, Xiao R (2012) Flavonoids protect cerebrovascular endothelial cells through Nrf2 and PI3K from β-amyloid peptide-induced oxidative damage. Curr Neurovasc Res 9(1):32–41

Yang D, Liu J, Tian C, Zeng Y, Zheng YH, Fang Q, Li HH (2010) Epigallocatechin gallate inhibits angiotensin II-induced endothelial barrier dysfunction via inhibition of the p38 MAPK/ HSP27 pathway. Acta Pharmacol Sin 31:1401–1406

Zecca L, Youdim MB, Riederer P, Connor JR, Crichton RR (2004) Iron, brain ageing and neurodegenerative disorders. Nat Rev Neurosci 5(11):863–873

Zhang ZX, Román GC (1993) Worldwide occurrence of Parkinson's disease: an updated review. Neuroepidemiology 12(4):195–208

Zhu M, Rajamani S, Kaylor J, Han S, Zhou F, Fink AL (2004) The flavonoid baicalein inhibits fibrillation of alpha-synuclein and disaggregates existing fibrils. J Biol Chem 279(26):26846–26857

The Effect of (−)-Epigallo-catechin-(3)-gallate on Amyloidogenic Proteins Suggests a Common Mechanism

7

Kathrin Andrich and Jan Bieschke

Abstract

Studies on the interaction of the green tea polyphenol (−)-Epigallocatechin-3-gallate (EGCG) with fourteen disease-related amyloid polypeptides and prions Huntingtin, Amyloid-beta, alpha-Synuclein, islet amyloid polypeptide (IAPP), Sup35, NM25 and NM4, tau, MSP2, semen-derived enhancer of virus infection (SEVI), immunoglobulin light chains, beta-microglobulin, prion protein (PrP) and Insulin, have yielded a variety of experimental observations. Here, we analyze whether these observations could be explained by a common mechanism and give a broad overview of the published experimental data on the actions of EGCG. Firstly, we look at the influence of EGCG on aggregate toxicity, morphology, seeding competence, stability and conformational changes. Secondly, we screened publications elucidating the biochemical mechanism of EGCG intervention, notably the effect of EGCG on aggregation kinetics, oligomeric aggregation intermediates, and its binding mode to polypeptides. We hypothesize that the experimental results may be reconciled in a common mechanism, in which EGCG binds to cross-beta sheet aggregation intermediates. The relative position of these species in the energy profile of the amyloid cascade would determine the net effect of EGCG on aggregation and disaggregation of amyloid fibrils.

K. Andrich
Department of Biomedical Engineering, Washington University in St. Louis, Saint Louis, MO, USA

Max Delbrück Center for Molecular Medicine, Berlin, Germany

J. Bieschke (✉)
Department of Biomedical Engineering, Washington University in St. Louis, Saint Louis, MO, USA
e-mail: bieschke@wustl.edu

N. Vassallo (ed.), *Natural Compounds as Therapeutic Agents for Amyloidogenic Diseases*, Advances in Experimental Medicine and Biology 863, DOI 10.1007/978-3-319-18365-7_7

Keywords
Epigallocathechin-3-gallate (EGCG) • Amyloid polypeptides • Aggregation • Fibrils

7.1 Introduction

The polyphenol (−)-Epigallocatechin-3-gallate (EGCG) is produced by the tea plant (*Camellia sinensis*) and accounts for about 10 % of the dry weight of its leaves (Graham 1992). The flavonoid EGCG belongs to the subclass of flavan-3-ols which have an unsaturated C-ring with the B-ring attached to their C2-atom (Fig. 7.1a). In EGCG, the B-ring is attached in epi conformation and the 3-ol of the C-ring is substituted by an 3-O-gallate function (Beecher 2003). EGCG has been found to inhibit formation of amyloid fibrils and to bind to existing amyloid fibrils and remodel them into non-amyloid aggregates (Fig. 7.1b) (Ehrnhoefer et al. 2006, 2008; Bieschke et al. 2010). Its anti-aggregation mechanism has since been studied for 14 amyloidogenic peptides and proteins. At first glance, the data seem to suggest that EGCG has quite different effects on the different polypeptides.

Our review aims to compare these effects on a phenomenological and mechanistic level. To do so, we will concentrate on disease-related polypeptides and prions. In the first part, we will give a broad overview of the published experimental data on the action of EGCG, while in the second part we will discuss how the experimental results might be reconciled in a general mechanism that leads to the inhibition of amyloidogenic aggregation and the disaggregation of amyloid fibrils.

7.2 Phenomenological Overview of EGCG

About 10 years ago, it was first reported that green tea extract and EGCG reduced the cytotoxicity of the Amyloid-beta peptide (Aβ) involved in Alzheimer's Disease (AD) (Levites et al. 2003) and also reduced the amount and size of amyloid deposits in APP transgenic mice (Rezai-Zadeh et al. 2005). Likewise, it diminished the formation of toxic aggregates in yeast and *Drosophila* models of Huntingtin protein (Htt) aggregation (Ehrnhoefer et al. 2006). These early results prompted a large number of studies on the effect of EGCG on various amyloidogenic proteins. Tables 7.1, 7.2, and 7.3 present an overview of the effects of EGCG extracted from data that were published prior to August 2014. Empty fields mark characteristics for which no data are available for the respective polypeptide. Some characteristics have been addressed in an extensive number of studies; in these cases, the complete reference list is included in the overview table while selected studies are referenced in the text.

7.2.1 Amyloid Toxicity

Amyloid fibrils may have functional roles (Chapman et al. 2002; Berson et al. 2003; Fowler et al. 2006). Several functional prions that possess amyloid structural elements have been identified in yeast and other fungi (Shorter and Lindquist 2005; Coustou et al. 1997). However, in many polypeptides amyloid formation is toxic to cells. Indeed, deposition of misfolded polypeptides in amyloid or amyloid-like aggregates is characteristic of some of the most prominent neurodegenerative diseases: the Amyloid-beta peptides (Aβ) derived from the Amyloid Precursor Protein (APP) and the tau protein in Alzheimer's disease (AD) (Golde et al. 1993; Kosik et al. 1986), Huntingtin protein (Htt) in Huntington's disease (HD) (Trottier et al. 1995; Scherzinger et al. 1997), alpha-Synuclein (α-Syn) in Parkinson's disease (PD) (Takeda et al. 1998), and the prion protein PrP in transmissible spongiform encephalopathies (TSE) (Prusiner 1998). Amyloid deposits also form in systemic diseases: globular

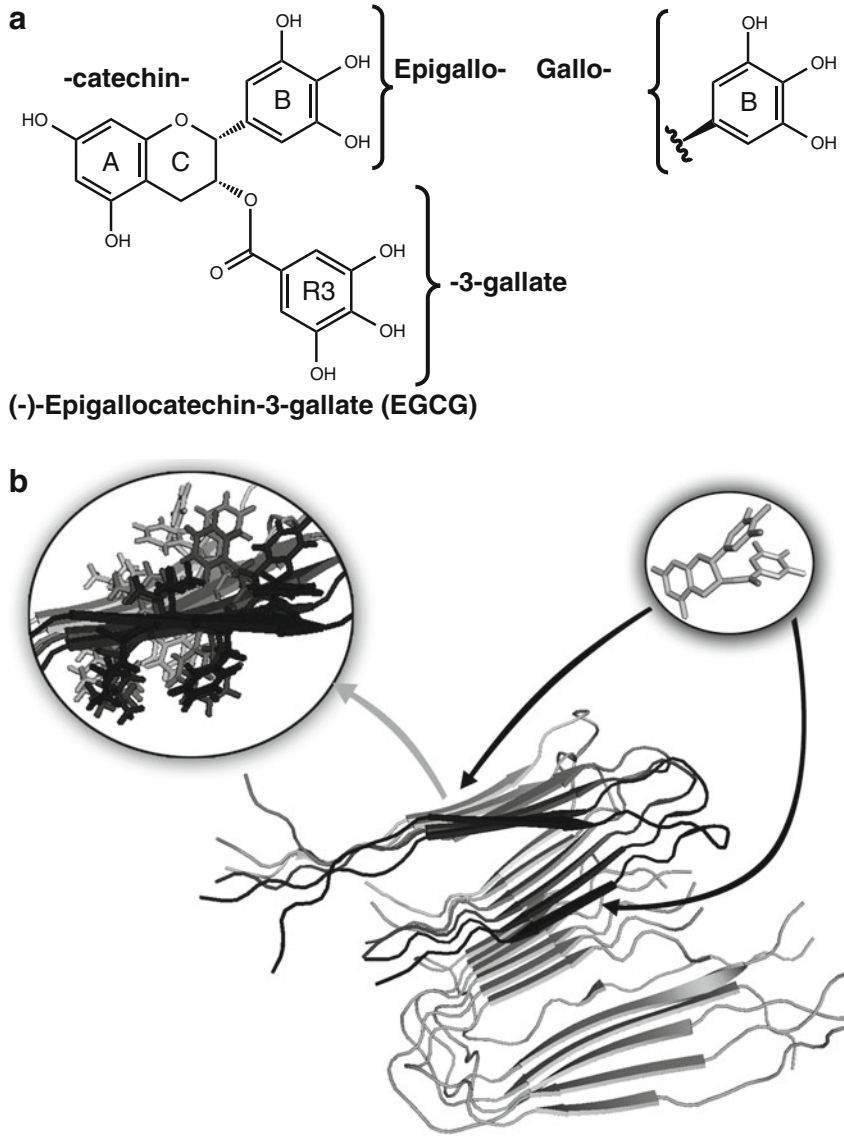


Fig. 7.1 (−)-Epigallocatechin-3-gallate (EGCG) and its interaction with Aβ amyloid fibrils (**a**) EGCG is a flavonoid of the subclass flavan-3-ol having an unsaturated C-ring with the B-ring attached to its C2-atom. In EGCG, the B-ring is attached in epi conformation and the 3-ol of the C-ring is substituted by a 3-O-gallate function. The phenyl rings are di- or trisubstituted by phenol groups, thus making EGCG a polyphenol (Beecher 2003). (**b**) In Aβ fibrils, the monomers form a hairpin structure, with the N-terminal domain facing the fibril surface, while the C-terminal domain is directed towards the fibril core. Two or three monomers build the fibril base. The monomers within the fibrils are stacked on top of each other with adjacent monomers building intramolecular cross-β-sheets along the fibril axis (Lu et al. 2013; Petkova et al. 2006; Luhrs et al. 2005; Colletier et al. 2011). EGCG binding is indicated by black arrows. EGCG likely binds along the LVFF-motif (enhanced with side chain structure) (Grelle et al. 2011; Lopez del Amo et al. 2012; Wang et al. 2012b). This figure was made using Pymol and structure coordinates were taken from PDB entry 2LMO (Petkova et al. 2006)

Table 7.1 Effect of EGCG on disease-related polypeptides and prions (part 1)

Influence of EGCG	Htt	Aβ	α-Syn	IAPP
Aggregate morphology				
Prevents fibrils/aggregate formation		5	12, 23	25
Redirects into small amorphous aggregates		Y		
Leads to monomer depletion				
Disaggregates fibrils	1	12, 21	23	25, 26, 28
Remodels into large amorphous aggregates		12, 21	12	25, 26, 28
Kinetics				
Slows ThT kinetics		5, 14, 16, 17	5, 12	25, 27, 28
Accelerates ThT kinetics		N (5)	N (5, 12)	N (25,27, 28)
Reduces or inhibits seeding-competence		5, 7, 18, 21	5, 12	25, 26
Inhibits prion propagation				
Structural				
Redirects into non-SDS-stable species	1	N (12)		
Redirects into semi-SDS-stable species		5, 16	5, 12	
Redirects into SDS-stable species		12, 14, 21	12, 14	
Reduces proteinase K-resistance				
Redirect into non-A11-specific oligomers		17	5	
Uncovers antibody binding epitope				
Reduces ThT/CR fluorescence amplitude		5, 12, 14, 16, 17, 21	5, 12	25, 27, 28
EGCG structure and binding				
Non-epi-gallo-moiety reduces efficiency	1	8, 12		
Gallate-moiety necessary for effectivity	1	8, 12		
Binds to oligomers		5	5	28
Direct remodeling		21, 12	12	
Interacts with specific residues		18		
Hydrophobic interactions		9, 18, 19		
H-Bonding		9, 19		
π-π stacking		18		N (26)
Schiff-base formation		N (9, 19, 21)		
Disulfide bridge				N (26)
Destruction of salt-bridges		N(9, 18, 19, 21)		
Toxicity				
Reduces membrane permeabilization		16, 20	20, 24	
Inhibits cytotoxicity (cell models)	1	10 papers[a]	5, 12, 24	25
Inhibits deposits in vivo (animal models)		3, 4, 6, 7, 10, 11, 15		

(continued)

Table 7.1 (continued)

Influence of EGCG	Htt	Aβ	α-Syn	IAPP
Reduces cognitive impairment in mice		4, 6		
Inhibits deposits in vivo (humans)				
Phase 2 or 3 clinical trial	Y	Y	Y	

N( ) indicates studies with negative results, blank spaces indicate that no experimental results are available
Aβ Amyloid-beta, *Htt* Huntingtin, *IAPP* Islet Amyloid Polypeptide, *α-Syn* alpha-Synuclein; *1* (Ehrnhoefer et al. 2006), *2* (Levites et al. 2003), *3* (Rezai-Zadeh et al. 2005), *4* (Rezai-Zadeh et al. 2008), *5* (Ehrnhoefer et al. 2008), *6* (Lee et al. 2009b), *7* (Lee et al. 2009a), *8* (Lin et al. 2009), *9* (Wang et al. 2010), *10* (Giunta et al. 2010), *11* (Abbas and Wink 2010), *12* (Bieschke et al. 2010), *13* (He et al. 2011), *14* (Grelle et al. 2011), *15* (Dragicevic et al. 2011), *16* (Gauci et al. 2011), *17* (Sinha et al. 2012), *18* (Lopez del Amo et al. 2012), *19* (Wang et al. 2012b), *20* (Camilleri et al. 2013), *21* (Palhano et al. 2013), *22* (Lee et al. 2013), *23* (Caruana et al. 2011), *24* (Lorenzen et al. 2014), *25* (Meng et al. 2010), *26* (Cao and Raleigh 2012), *27* (Suzuki et al. 2012), *28* (Young et al. 2014)
[a]2, 5, 8, 12, 13, 14, 15, 17, 18, 21

monoclonal immunoglobulin light chains (LC) and Transthyretin (TTR) deposits form in the heart and in other tissues in systemic Light Chain Amyloidosis (AL) (Solomon et al. 1982) and in Transthyretin-Amyloidosis, respectively (Saraiva et al. 1984). In Diabetes Mellitus type II (DM II) the Islet Amyloid Polypeptide (IAPP, amylin) forms amyloid deposits in the insulin producing islet-β-cells (Johnson et al. 1989). *In vitro*, it was observed that also insulin is capable of forming amyloid fibrils under physiological conditions, albeit at high concentrations of 345 μM (Wang et al. 2012a). Diabetes mellitus has large economic implications; about 347 million people worldwide suffer from DM type I and type II (Danaei et al. 2011), which dwarfs even prevalence of AD (estimated 44 million cases in 2013) (Prince et al. 2014).

Patients undergoing long-term hemodialysis are at risk of developing dialysis-related amyloidosis (DRA), where β_2-Microglobin (β2m) is deposited, a light chain of the major histocompatibility complex I (MHC-I) (Grey et al. 1973) that is prone to form amyloid deposits (Gejyo et al. 1985, Linke 1985).

Interestingly, two other devastating diseases are also indirectly connected to amyloid formation. An amyloid forming-protein derived from human semen was found to enhance the infectious potential of HIV and was therefore named semen-derived enhancer of virus infection (SEVI). The parasite plasmodium falciparum involved in malaria pathogenesis produces a highly abundant surface protein, the merozoite surface protein 2 (MSP2) which is considered as a candidate for a malaria vaccine (Genton et al. 2002). It was shown that MSP2 has also an amyloidogenic nature (Yang et al. 2007). While this is by no means a complete list of amyloid diseases, experimental data on EGCG have been collected for all of these polypeptides, illustrating the broad interest in EGCG that led to a large body of experimental data that are available for mechanistic analysis.

7.2.2 Reduction in Cytotoxicity of Amyloidogenic Proteins

EGCG reduced the toxicity of amyloidogenic polypeptides related to a number of neurodegenerative diseases and systemic diseases that were listed above. EGCG treatment reduced cytotoxicity of Aβ (Levites et al. 2003; Ehrnhoefer et al. 2008; Bieschke et al. 2010) [see also Table 7.1], Htt (Ehrnhoefer et al. 2006), α-Syn (Ehrnhoefer et al. 2008; Bieschke et al. 2010; Lorenzen et al. 2014), IAPP (Meng et al. 2010) and tau (Wobst et al. 2015) in cellular models. Experiments probing the effect of EGCG on TTR toxicity in cell culture were inconclusive (Miyata et al. 2010).

Amyloid toxicity is believed to be linked to the presence of oligomeric aggregation intermediates (Walsh et al. 2002a; Haass and Selkoe 2007).

Table 7.2 Effect of EGCG on disease-related and polypeptides and prions (part 2)

Influence of EGCG	Sup35 NM25	Sup35 NM4	tau	MSP2	SEVI
Aggregate morphology					
Prevents fibrils/aggregate formation	30	N (30)	31		34, 36
Redirects into small amorphous aggregates			31		
Leads to monomer depletion					
Disaggregates fibrils	30	N (30)	31	33	34
Remodels into large amorphous aggregates	30	N (30)		33	34
Kinetics					
Slows ThT kinetics			31	32, 33	34
Accelerates ThT kinetics			N (31)	N (32, 33)	N (34)
Reduces or inhibits seeding-competence	30	N (30)			
Inhibits prion propagation	30	N (30)			
Structural					
Redirects into non-SDS-stable species					
Redirects into semi-SDS-stable species					
Redirects into SDS-stable species				32, 33	
Reduces proteinase K-resistance					
Redirect into non-A11-specific oligomers			31		
Uncovers antibody binding epitope			31		
Reduces ThT/CR fluorescence amplitude	30	30	31	32, 33	34
EGCG structure and binding					
Non-epi-gallo-moiety reduces efficiency					34, 36
Gallate-moiety necessary for effectivity	30	30			34, 36
Binds to oligomers			31	32	34
Direct remodeling					
Interacts with specific residues				32	34
Hydrophobic interactions				32	
H-bonding					
π-π stacking					
Schiff-base formation				N (32)	−34
Disulfide bridge				N (32)	
Destruction of salt-bridges					
Toxicity					
Reduces membrane permeabilization					
Inhibits cytotoxicity (cell models)			31		
Inhibits deposits in vivo (animal models)					
Reduces cognitive impairment in mice					
Inhibits deposits in vivo (humans)					
Phase 2 or 3 clinical trial					

N( ) indicates studies with negative results; blank spaces indicate that no experimental results are available
Sup35 NM25 prion strain (head region 21–38, center region 39–90, tail region 91–106), *Sup35 NM4* prion strain (head region 21–38, center region 39–78, tail region 79–96), *tau* HisK18ΔK280 fragment of tau protein, *MSP2* Plasmodium falciparum merozoite surface protein 2, *SEVI* semen-derived enhancer of virus infection , *30* (Roberts et al. 2009), *31*(Wobst et al. 2015), *32* (Chandrashekaran et al. 2010), *33* (Chandrashekaran et al. 2011), *34* (Hauber et al. 2009), *36* (Popovych et al. 2012)

Table 7.3 Effect of EGCG on disease-related polypeptides and prions (part 3)

Influence of EGCG	LC	TTR	β2-m	PrP	Ins
Aggregate morphology					
Prevents fibrils/aggregate formation		43	N (49)		51
Redirects into small amorphous aggregates		44			51
Leads to monomer depletion		43			N (51)
Disaggregates fibrils					
Remodels into large amorphous aggregates		44			
Kinetics					
Slows ThT kinetics	N (41)		49		
Accelerates ThT kinetics	41		N (49)		
Reduces or inhibits seeding-competence				50	
Inhibits prion propagation					
Structural					
Redirects into non-SDS-stable species	N (41)				
Redirects into semi-SDS-stable species	41	43			
Redirects into SDS-stable species	N (41)				
Reduces proteinase K-resistance				50	
Redirect into non-A11-specific oligomers					
Uncovers antibody binding epitope					
Reduces ThT/CR fluorescence amplitude	41	43	49		
EGCG structure and binding					
Non-epi-gallo-moiety reduces efficiency					
Gallate-moiety necessary for effectivity				50	
Binds to oligomers		43			
Direct remodeling					
Interacts with specific residues					
Hydrophobic interactions					
H-bonding					
π-π stacking					
Schiff-base formation					
Disulfide bridge					
Destruction of salt-bridges					
Toxicity					
Reduces membrane permeabilization					
Inhibits cytotoxicity (cell models)					
Inhibits deposits in vivo (animal models)		45, 47			
Reduces cognitive impairment in mice					
Inhibits deposits in vivo (humans)	37, 38	46			
Phase 2 or 3 clinical trial	Y	Y			

N( ) indicates studies with negative results; blank spaces indicate that no experimental results are available
LC immunoglobulin light chain protein, *TTR* transthyretin (studies with variants: wt/V30M/E54K/Y38F/L55P), *β2-m* β_2-Microglobulin, *PrP* prion protein, *Ins* Insulin *37* (Mereles et al. 2008), *38* (Mereles et al. 2010), *41* (unpublished data), *43* (Miyata et al. 2010), *44* (Ferreira et al. 2011), *45* (Ferreira et al. 2012b), *46* (Kristen et al. 2012), *47* (Ferreira et al. 2012a), *49* (Woods et al. 2011), *50* (Rambold et al. 2008), *51* (Wang et al. 2012a)

However, the mechanism of toxicity has not yet been completely clarified. For Aβ and α-Syn, it was found that aggregates can permeabilize vesicular and mitochondrial membranes (Rodrigues et al. 2000; Volles et al. 2001). EGCG was shown to inhibit permeabilization of model membranes and mitochondrial membranes (Gauci et al. 2011; Caruana et al. 2011; Camilleri et al. 2013). In an *ex vivo* study with human semen samples from 47 individuals, the majority of samples contained SEVI species and EGCG was able to efficiently inhibit the SEVI-mediated HIV activity (Hauber et al. 2009).

7.2.3 Reduction of Amyloid Deposits and Toxicity in Higher Organisms

EGCG was shown to reduce amyloid deposition in animal models of protein misfolding disorders. Treatment with green tea extract rich in EGCG lowered the load of amyloid aggregates in *Caenorhabditis elegans* (Abbas and Wink 2010) and in AD mouse models (Rezai-Zadeh et al. 2005; Giunta et al. 2010; Dragicevic et al. 2011) [see also Table 7.1], where it also reduced cognitive impairment (Rezai-Zadeh et al. 2008; Lee et al. 2009b). Depletion of amyloid deposits was observed in mouse models of Transthyretin Amyloidosis (Ferreira et al. 2012a, b), while depletion of amyloidogenic deposits in the human heart was observed in a phase II clinical trial after EGCG treatment (Kristen et al. 2012). Treatment with EGCG of a case of AL-Amyloidosis after several unsuccessful cycles of chemotherapy resulted in reduction of amyloid deposits in the patients' heart (Mereles et al. 2008). The same group performed a retrospective study on the influence of green tea and EGCG consumption on amyloid deposition in AL patients (Mereles et al. 2010). Phase II clinical studies by two European AL-Amyloidosis Treatment Centers are currently in progress (Schönland 2013; Merlini 2013). Phase II clinical studies have also been performed on AD, PD and HD (Chan 2007; Friedemann and Dörr 2009; Priller 2011).

7.2.4 Pharmacological Aspects of Aggregation Inhibition

Therapeutic use of EGCG is complicated by several pharmacokinetic drawbacks, most notably the widely variable bioavailability after oral consumption (Hunstein 2007). Nevertheless, it may be a treatment option worth evaluating, especially in rare diseases that are otherwise unprofitable for drug development. Exchanging the B-ring epigallo-moiety (Beecher 2003) by a gallo-moiety (Fig. 7.1) reduced the efficiency of the catechin on Htt aggregation (Ehrnhoefer et al. 2006), on Aβ aggregation (Bieschke et al. 2010) and Aβ cytotoxicity (Lin et al. 2009), and on SEVI-mediated infectivity (Hauber et al. 2009; Popovych et al. 2012). Depleting the C3-atom gallate function from the C-Ring showed comparable effects in the mentioned studies. In contrast to EGCG, Epigallocatechin (EGC) was neither able to disaggregate fibrils of the Sup35 NM25 strains prionogenic N-terminal domain (NM25) nor to inhibit its prion propagation (Roberts et al. 2009). EGCG treatment led to lysosomal degradation of PrP^{C} and subsequent accumulation of PrP^{Sc} in transiently transfected cell lines. While EGCG efficiently interfered with the PrP^{Sc} accumulation, four times the amount of EGC was necessary to achieve the same effect (Rambold et al. 2008).

7.3 Changes in Aggregate Morphology of Amyloid Precursors and Amyloidogenic Species

7.3.1 Folding State of Amyloid Precursors and Amyloidogenic Species

Protein misfolding can result in the formation of rope-like or straight amyloid fibrils that have highly stable cross-β sheet structures, which are aligned along the fibril axis (Chiti and Dobson 2006). The amyloidogenic species could either be a fragment of a precursor polypeptide like Aβ,

Htt-exon1 and Insulin (Permutt et al. 1981), or entire proteins like α-Syn (Ulmer et al. 2005), LC protein (Redegeld and Nijkamp 2003) and TTR (Wojtczak et al. 1992). The native structure of amyloid precursors range from highly unstructured proteins like Htt (Zhang et al. 2013) and α-Syn (Dedmon et al. 2005) to highly structured β-sheet rich proteins like β2m (Bjorkman et al. 1987), TTR (Wojtczak et al. 1992) and LC (Edmundson et al. 1993), or α-helix rich proteins like the PrP (90–232) (Riek et al. 1996).

Irrespective of the starting point, the formation of the cross-β-sheet structure requires at least a partial unfolding or structural rearrangement of the amyloid precursor. The presence of EGCG during aggregation reduced the β-sheet content of all the protein aggregates for which secondary structure was measured, namely α-Syn (Ehrnhoefer et al. 2008; Bieschke et al. 2010), MSP2 (Chandrashekaran et al. 2010, 2011), PrP (Rambold et al. 2008) and Insulin (Wang et al. 2012a).

7.3.2 Redirection of Aggregation into Non-amyloidogenic Species

The question of whether EGCG prevents the formation of amyloidogenic fibrils and aggregates was addressed in most studies with amyloidogenic polypeptides for which EGCG data was available. Its effect on *de novo* amyloid formation depends on the amyloidogenic protein (Fig. 7.2a). In most proteins, EGCG prevented amyloid fibril formation, and redirected the assembly process to generate amorphous aggregate species. This was observed for Htt (Ehrnhoefer et al. 2006), Aβ (Ehrnhoefer et al. 2008; Lopez del Amo et al. 2012), α-Syn (Bieschke et al. 2010; Suzuki et al. 2012), the HIV-mediating SEVI (Hauber et al. 2009; Popovych et al. 2012), IAPP (Meng et al. 2010; Suzuki et al. 2012), and Insulin (Wang et al. 2012a). EGCG was on the other hand unable to prevent fibril formation by β2m (Woods et al. 2011), the prion protein (Rambold et al. 2008), or heparin-induced fibril formation of tau (Wobst et al. 2015). However, EGCG completely prevented the formation of β-sheet rich aggregates of an aggregation-prone mutant tau (His-K18ΔK280) in the absence of heparin (Wobst et al. 2015). The crucial rate limiting step in TTR-amyloid formation involves monomerization of the TTR tetramer (Hammarstrom et al. 2003). Here too, EGCG redirected the aggregation of mutant TTR-L55P and TTR-Y78F from large amorphous aggregates into small amorphous aggregates (Ferreira et al. 2011). In another study, it prevented fibril formation of wt-TRR and TTR-V30M by preventing the monomerization of TTR (Miyata et al. 2010).

Remarkably the fibril formation of Sup35 NM25 was also redirected into amorphous aggregates, while fibril formation was not impaired by EGCG in the NM4 prion N-terminal domain (Roberts et al. 2009).

7.3.3 Remodeling of Pre-formed Amyloid Fibrils into Non-amyloidogenic Species

As discussed above, it is established that EGCG generally redirects the aggregation of an amyloid precursor. One could therefore ask whether it can also disrupt pre-formed amyloid fibrils, and whether such a disruption would result in similar supramolecular assemblies to those observed in aggregation in the presence of EGCG. Disaggregation of amyloid fibrils into comparable amorphous aggregate species was in fact reported in presence of EGCG for Aβ (Bieschke et al. 2010; Palhano et al. 2013), α-Syn (Bieschke et al. 2010; Caruana et al. 2011), tau His-K18ΔK280 (Wobst et al. 2015), IAPP (Meng et al. 2010; Cao and Raleigh 2012; Young et al. 2014), SEVI (Hauber et al. 2009) and the variants TTR-L55P and TTR-Y38F (Ferreira et al. 2011). Even fibrils of Sup35 were either disrupted and remodeled into large amorphous aggregates by EGCG (NM25), or not altered (NM4) corresponding to the results of *de novo* aggregation in presence of EGCG (Roberts et al. 2009). Additionally, EGCG was able to remodel preformed fibrils of MSP2 into large amorphous aggregates (Chandrashekaran et al. 2011).

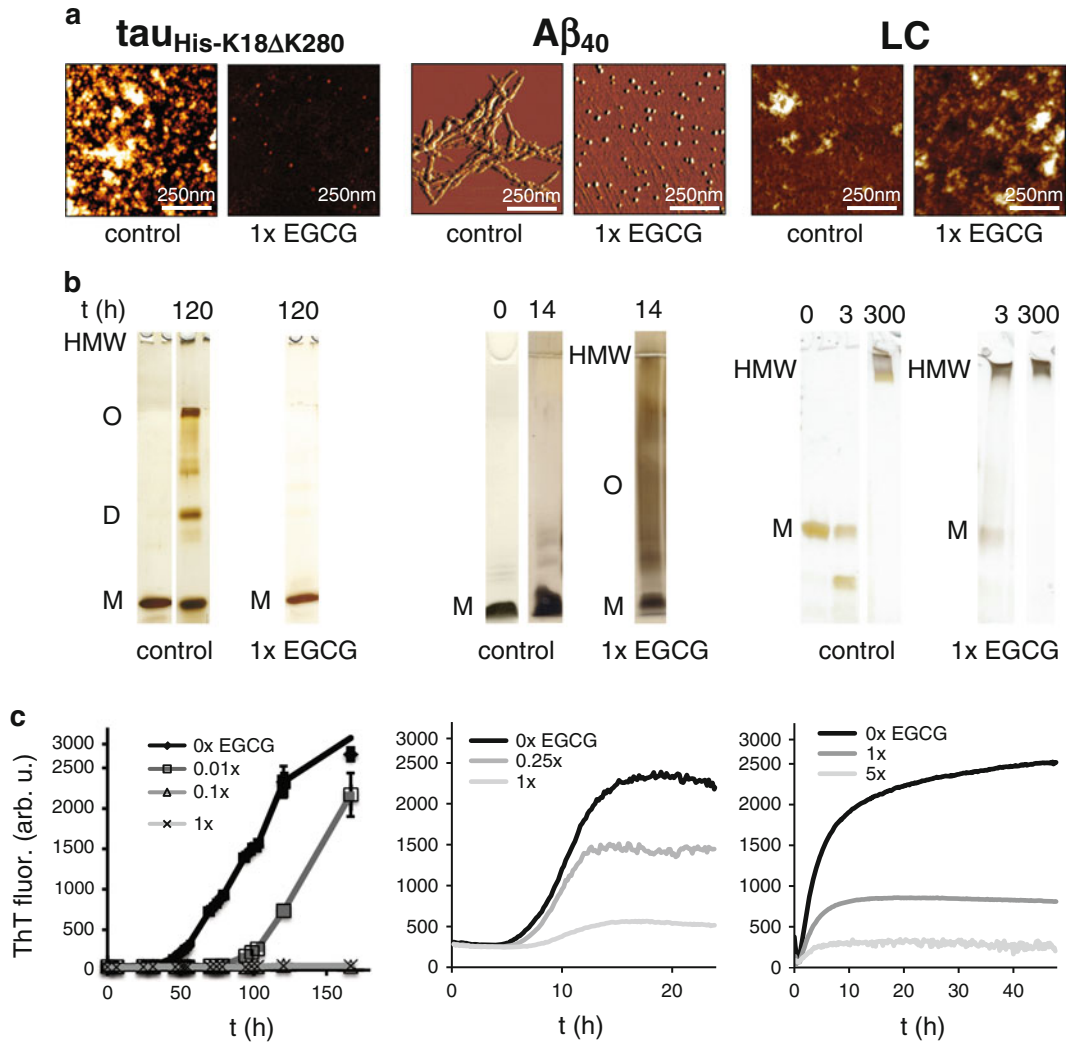


Fig. 7.2 Influence of EGCG on aggregation kinetics, aggregate morphology and SDS-stability of tau, Aβ and LC. (**a**) Aggregation kinetics in absence (*black*) and presence (*gray*) of EGCG, where 1× mean equimolar concentrations of the amyloid precursor and EGCG. (**b**) Aggregate morphology after aggregation in absence and presence of EGCG. (**c**) SDS-stability (tau, Aβ) and semi-SDS-stability (LC, samples were neither reduced nor boiled) of aggregates formed in absence and presence of EGCG. With tau, EGCG prevents formation of SDS-stable aggregates while with Aβ it leads to SDS-stability indicating structural changes induced by EGCG. In LC, the formation of semi-SDS-stable aggregates was accelerated

7.3.4 Seeding Competence of EGCG-Induced Aggregates

A hallmark of amyloidogenic aggregates is their ability to incorporate monomeric polypeptides into amyloid structures. This process is referred to as 'seeding-competence', which is also a key property in prion propagation (Jarrett and Lansbury 1993). A simple test for seeding competence is to compare aggregation kinetics in the presence of a suspected amyloid precursor to those of unseeded aggregation (Ehrnhoefer et al. 2008). In mechanisms where nucleation is the rate-limiting step, the addition of seeding- or propagation-competent species will shorten or abrogate the lag phase.

Aggregates that resulted either from redirected monomer aggregation or from remodeling of

mature fibrils by EGCG were either seeding-incompetent or showed reduced seeding-competence of Aβ (Ehrnhoefer et al. 2008; Bieschke et al. 2010; Palhano et al. 2013), α-Syn (Ehrnhoefer et al. 2008; Bieschke et al. 2010) and IAPP (Meng et al. 2010; Cao and Raleigh 2012). The prion protein (Rambold et al. 2008) as well as the yeast prion NM25 were also transformed into propagation-incompetent species, while the propagation-competence of NM4 was not impaired (Roberts et al. 2009).

7.3.5 Altered Aggregate Stability and Conformation

Resistance against denaturation by boiling in the presence of Sodium Dodecyl Sulfate (SDS-stability) is a rapid and useful indicator of aggregate stability (Wanker et al. 1999). Upon fibril formation, many amyloidogenic polypeptides become resistant to SDS denaturation and to proteolytic digestion by proteinase-K (PK-resistance). Changes in both properties would indicate structural changes in the aggregation process, although the structural basis of these effects is poorly understood. The SDS-stability can be measured either by a filter retardation assay (FRA) (Wanker et al. 1999) or SDS-PAGE (Ehrnhoefer et al 2008, Bieschke et al 2010). The latter is performed in the presence of SDS but without heating or at a temperature gradient, and can resolve differences in stability more sensitively. It is worth noting that apparent molecular weight in (semi-) denaturing gels does not necessarily reflect the size of the aggregates in solution.

The response of different amyloidogenic proteins to EGCG with respect to SDS and PK-stability varies considerably (Fig. 7.2b). EGCG prevented the formation of SDS-stable aggregates of Huntingtin (Ehrnhoefer et al. 2006) and an aggregation-prone tau mutant His-K18ΔK280, (Wobst et al. 2015). In contrast, EGCG induced highly SDS-stable aggregates of Aβ and α-Syn (Ehrnhoefer et al. 2008; Bieschke et al. 2010; Grelle et al. 2011; Gauci et al. 2011; Palhano et al. 2013). Similarly, we observed that EGCG accelerates the formation of SDS-stable species of immunoglobulin light chains (LC, Fig. 7.2b). SDS-stable aggregates of Aβ and α-Syn are structurally different from SDS-resistant fibrils these polypeptides form in the absence of EGCG. In LC aggregation, EGCG accelerates the formation of semi-SDS-stable aggregates. MSP2 was also reported to produce SDS-stable oligomer species in the presence of EGCG (Chandrashekaran et al. 2010, 2011). Rambold et al. (2008) found that EGCG reduced the PK-resistance of infectious PrP aggregates.

7.3.6 Conformational Change Can Be Probed by Antibody Binding

The anti-oligomer antibody A11 recognizes a common intermediate oligomeric structure in Aβ40 and Aβ42 (Kayed et al. 2003), Sup35 (Shorter and Lindquist 2004), β2m (Ribeiro et al. 2012), α-Syn (Ehrnhoefer et al. 2008) and tau (Flach et al. 2012; Wobst et al. 2015). EGCG prevented the binding of A11 to Aβ (Sinha et al. 2012), α-Syn (Ehrnhoefer et al. 2008) and tau (Wobst et al. 2015) indicating that either structural changes in the protein aggregates or EGCG binding obscured the antibody binding sites.

7.4 Towards a Holistic Molecular Mechanism for EGCG-Amyloid Interaction

7.4.1 Altered Protein Aggregation Kinetics by EGCG

The benzothiazole dye Thioflavin T (ThT) binds amyloid, amyloid fibrils and cross-β-sheet aggregation intermediates with a characteristic red-shift of its emission spectrum, which does not occur in presence of unstructured monomers or oligomers (LeVine 1997). Therefore, it is frequently used to observe the aggregation kinetics leading to formation of amyloid fibrils by numerous amyloidogenic polypeptides (LeVine 1999).

EGCG reduced the ThT amplitude in most amyloidogenic polypeptides in a dose-dependent

manner (see Table 7.1). This could be either due to a change in aggregate morphology, to partial sequestration of the amyloidogenic polypeptide or to an inhibition of ThT binding by competitive EGCG binding. After aggregation under influence of EGCG, the ThT fluorescence of Sup35 prion strains and TTR was reduced (Roberts et al. 2009; Miyata et al. 2010). By measuring kinetics of ThT fluorescence, it was shown that EGCG also slowed down the aggregation kinetics of Aβ (Ehrnhoefer et al. 2008; Grelle et al. 2011; Gauci et al. 2011; Sinha et al. 2012), α-Syn (Ehrnhoefer et al. 2008; Bieschke et al. 2010), IAPP (Meng et al. 2010; Suzuki et al. 2012; Young et al. 2014), tau (Wobst et al. 2015), MSP2 (Chandrashekaran et al. 2010, 2011), SEVI (Hauber et al. 2009) and β2m (Woods et al. 2011). These findings suggest that inhibition of aggregation kinetics might be due to a general mechanism of action of EGCG. EGCG however did not affect the kinetics of fibril formation of the tau protein when this was induced by heparin (Wobst et al. 2015). This is in contrast to the efficiency of EGCG in inhibiting the formation of ThT-positive tau oligomers in the absence of heparin, even at high substoichiometric concentrations (Fig. 7.2c) (Wobst et al. 2015).

When examining the effect of EGCG on different monoclonal immunoglobulin light chains isolated from urine of patients suffering either from AL Amyloidosis (clinical amyloid deposits) or from Multiple Myeloma (no clinical amyloid deposits), the formation of ThT-positive species was observed for all light chains (Andrich and Bieschke 2014). Typical aggregation kinetics showed a rapid phase of less than two hours followed by a slow phase of several days. In the presence of EGCG, only the ThT signal during the slow aggregation phase was reduced (Fig. 7.2c). The fact that light chains from patients without clinical amyloid deposits showed also the formation of ThT-positive species suggests that not every ThT-positive species of LC necessarily forms amyloid fibrils in patients.

We will discuss below how these different effects of EGCG on aggregation kinetics – inhibition, neutral, or acceleration – might be reconciled within a single model.

7.4.2 EGCG Interacts with Oligomeric Amyloid Precursors

EGCG binds to oligomeric species of Aβ, α-Syn and tau (Ehrnhoefer et al. 2008; Wobst et al. 2015). EGCG binding to oligomers was also reported for IAPP (Young et al. 2014), MSP2 (Chandrashekaran et al. 2010) and SEVI (Hauber et al. 2009).

A more difficult question to answer is, whether EGCG is able to bind to monomeric amyloid precursors. Nitro blue tetrazolium chloride (NBT) is a dye that can be used to stain EGCG (Paz et al. 1991). In aggregation assays, EGCG-specific staining by NBT was found for Aβ and α-Syn, and tau species that ran as monomers on SDS-PAGE (Ehrnhoefer et al. 2008). However, peptide species running as monomers on an SDS gel are not necessarily monomeric in solution. SDS-labile oligomers could bind EGCG and still disassemble during SDS-PAGE. These species would be distinct from monomers within the aggregation cascade. EGCG binding to native proteins, such as albumin has been observed at higher stoichiometric ratios (Ehrnhoefer et al. 2008; Bae et al. 2009; Nozaki et al. 2009).

EGCG binding to the aggregation-prone tau mutant His-K18ΔK280 resulted in monomers with a slightly lower electrophoretic mobility than the monomers prior aggregation, however the sub-stoichiometric effect of EGCG makes it likely that it preferentially binds to oligomeric aggregation intermediates rather than monomeric proteins (Wobst et al. 2015). Similarly, EGCG binding to the monomeric Aβ peptide was observed at a broad range of stoichiometries (Wang et al. 2010, 2012b), suggesting that multiple binding modes of EGCG exist to the Aβ peptide, possibly with a high affinity binding to oligomeric aggregation intermediates and lower affinity binding to the monomeric peptide. We postulate that the former high affinity interaction, rather than the latter low affinity interaction, is responsible for the inhibitory effect of EGCG on amyloid formation (Fig. 7.3a).

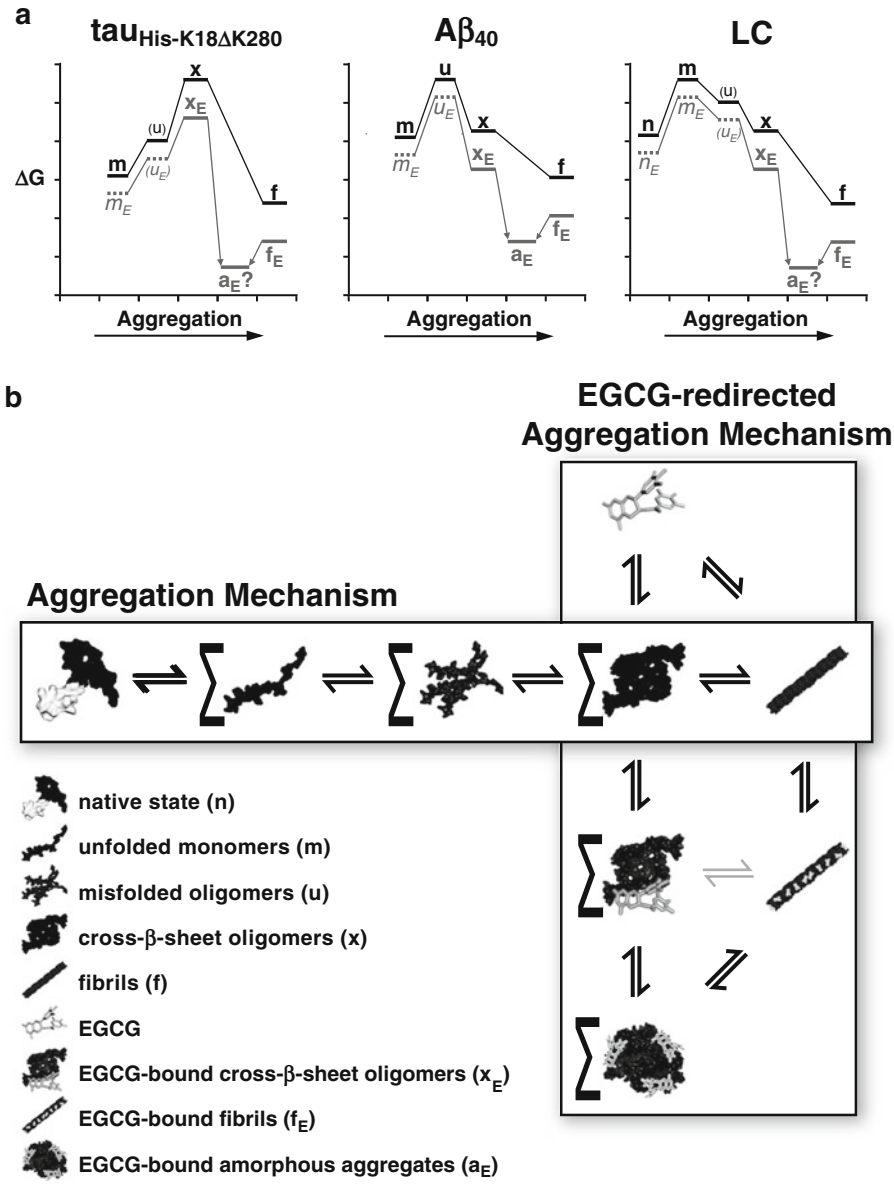


Fig. 7.3 Redirection of amyloid formation by EGCG. (**a**) Simplified two-dimensional energy diagrams for the aggregation in absence (*black*) and presence (*gray*) of EGCG, with *n* indicating the native state, *m* partially unfolded monomers, *u* misfolded oligomers, *x* cross-β-sheet oligomers, *f* fibrils and the subscript *E* indicating an EGCG-bound state. Dashed energy levels imply that EGCG binds only with low affinity, for bracketed intermediates it is unclear whether they exist in a non-transient manner, question marks refer to species for which the stability in respect to the fibrillar state is not clear. (**b**) The formation of amyloid can be summarized in a common mechanism. Different intermediate subspecies are denoted by a summation sign. We propose that EGCG binds preferably to the cross-β-sheet oligomers and the cross-β-sheet motif on the fibril surface in the aggregation cascade. Hence, EGCG disturbs the highly ordered fibril structure and induces the formation of EGCG-bound amorphous aggregates

7.4.3 EGCG Remodels Aβ Fibrils Without Releasing Monomers or Oligomers

In the case of the Aβ peptides, it is well established that oligomeric intermediate species are cytotoxic (Walsh et al. 2002b; Haass and Selkoe 2007). We found that EGCG remodels fibrillar Aβ into spherical aggregates that are indistinguishable from those formed by monomeric Aβ in the presence of EGCG (Bieschke et al. 2010). Correspondingly, the remodeled aggregates exhibit reduced cytotoxicity. This would suggest that no toxic oligomeric species are produced during the remodeling of Aβ by EGCG. This hypothesis was tested by generating Aβ fibrils, each labeled by incorporating Aβ monomers that were linked either to green or red fluorophores. Mixtures of fibrils with both labels were incubated in presence of EGCG and the remodeling of fibrils was observed using fluorescence microscopy. If the remodeling process had involved the random dissociation of fibrils it would have resulted in oligomers in which red- and green-labeled monomers were statistically distributed. Instead, we observed that oligomers were clustered into either predominantly green or red, indicating that they had resulted from the remodeling of individual fibrils. Therefore, we concluded that EGCG directly remodels Aβ fibrils rather than disaggregating them into monomeric peptides (Bieschke et al. 2010). These findings are supported by Palhano et al. (2013), who were unable to detect by mass spectrometry Aβ40 monomers during remodeling.

7.4.4 Elucidating the Binding Mode of EGCG to Amyloid Fibrils and Precursors

7.4.4.1 Investigations of the Binding Mode of EGCG

A number of possible non-covalent and covalent binding modes for EGCG to misfolded polypeptides have been postulated: non-site specific interaction with exposed hydrophobic surfaces through the hydrophobic effect (Ehrnhoefer et al. 2008), site-specific hydrogen bonding (H-bonding) (Maiti et al. 2006), aromatic π-π-stacking (Scheraga et al. 1962), site-specific covalent binding, such as formation of disulfide-bridges via cysteines (Lambert et al. 2008) and formation of Schiff bases via primary amines (Ishii et al. 2011). EGCG binding to amyloidogenic polypeptides has been analyzed using various biophysical and biochemical methods. For instance, isothermal titration calorimetry (ITC) was used to measure overall binding energies (Wang et al. 2010, 2012b). This approach however depends on highly homogenous preparations of interaction partners, which would be challenging when measuring interaction of EGCG with oligomeric amyloid precursors. For that reason, studies have so far been limited to monomeric proteins and peptides (Wang et al. 2010; Li et al. 2013). Peptide fragments have been engineered to address the question of site-specific binding (Wang et al. 2012b).

This approach must however, be interpreted with caution, since the results are only applicable to the native full-length polypeptide if the structure of the fragments resembles the structure of the parent polypeptide or if EGCG binds independently of the secondary structure formation. While monomeric Aβ, as many other amyloidogenic polypeptides, is unstructured as a monomer, this is not necessarily true for oligomeric aggregation intermediates.

Single amino acids have been substituted or chemically modified in Aβ and IAPP to analyze site-specific interaction of EGCG with the respective amino acids. Specifically, lysines were removed to probe for Schiff base formation (Wang et al. 2010, Wang et al. 2012b, Palhano et al. 2013), phenylalanines and tyrosines for π-π-stacking (Cao and Raleigh 2012, Lopez del Amo et al. 2012) and cysteins for SH-linkage (Cao and Raleigh 2012). Of course, this approach also relies on the assumption that the substitution does not alter the structure of the polypeptide within the amyloid oligomers and fibrils.

Solution-state and, more so, solid-state NMR spectroscopy provide technically challenging, but non-disruptive methods for probing the structure of EGCG-bound polypeptides (Lopez del Amo

et al. 2012). Here, the solubility and size of the amyloid polypeptide as well as sample homogeneity and spectral resolution are limiting factors. Since all these methods have their constraints, it is necessary to combine the findings of different methods to draw a reliable picture.

7.4.4.2 EGCG is Binding to Cross-β-Sheet Motif in Aβ End-Stage Oligomers and Fibrils

Solid-state NMR (Lu et al. 2013; Petkova et al. 2006), X-ray crystallography (Colletier et al. 2011) as well as hydrogen-deuterium (H/D) exchange and mutational analysis (Luhrs et al. 2005) have elucidated Aβ fibrillar structure. Within the core of a fibril strand, the Aβ monomers form a hairpin structure (Fig. 7.1b). These hairpins are stacked on top of each other, in a way that peptide groups of adjacent monomers form intramolecular cross-β-sheet H-bonds while their amino acid side chains alternate towards the fibril surface and towards the fibril center. The side chains between the arms of the hairpins interdigitate to further stabilize the fibril structure (Colletier et al. 2011).

The outwardly directed N-terminal β-sheet region of Aβ contains an LVFF-motif with Leu17 and Phe19 orientated towards the inward C-terminal β-sheet region, while Val18 and Phe20 point out towards the fibril surface. Lysine16 could in principle provide a Schiff-base anchor directly upstream to the LVFF-motif. The N-terminal amino acids 1–14 of Aβ are believed to be mostly unstructured and not part of the cross-β-sheet structure (Luhrs et al. 2005, Petkova et al. 2006). The N-terminal tail of Aβ contains a high degree of aromatic, acidic and basic amino acids, which could interact with EGCG through π-π-stacking or H-bonding. In contrast, hydrophobic side chains are concentrated on the C-terminus of the peptide that lies on the second arm of the hairpin (Petkova et al. 2006).

The structure of EGCG-induced Aβ oligomers was examined using solid-state NMR spectroscopy, which also yielded insights towards the binding model of EGCG (Lopez del Amo et al. 2012). As expected, no clear structure could be observed for the flexible N-terminal domain. In contrast, the LVFF motif was well-structured in the presence of EGCG. However, its structure was distinct from that in the absence of EGCG and the β-sheet structure of the KLVFFA region was lost in the presence of EGCG, while the β-sheet on the C-terminus of Aβ remained intact. Additionally the rotation of the phenyl rings (Phe19/20) as well as the rotation of adjacent His13/14 was hindered upon EGCG binding, strongly suggesting an interaction via π-π-stacking between the outwardly directed rings and the aromatic rings of EGCG. A cross-correlation for Phe19/Leu34 was found in presence and absence of EGCG, suggesting that the hydrophobic zipper stabilizing the hairpin structure may be partly intact in presence of EGCG.

Data stemming from ITC experiments conducted by Wang et al. (2010, 2012b) suggest that EGCG is forming H-bonds with the N-terminal domain of Aβ whilst binding to the LVFF motif via hydrophobic interactions. The authors proposed that the mode of EGCG binding gradually shifts from H-bonding to hydrophobic interaction at higher binding stoichiometries, which remains to be confirmed using complementary methods. The mean binding energies found in these ITC studies are lower than would be expected for the formation of covalent bonds, such as Schiff bases.

EGCG binding to Aβ40 via Schiff base formation was specifically investigated by acetylation of the two lysine residues Lys16 and Lys28 (Palhano et al. 2013) Lysine acetylation did not alter the effect of EGCG on ThT fluorescence, seeding competence or toxicity. Acetylation of Aβ results in blocking of Lys16 and Lys28 and prevents the salt-bridge formation of Aps23/Lys28 that is present in Aβ fibrils (Luhrs et al. 2005). Lys16 and Lys28 were mutated into arginine to prevent Schiff base formation, but and at the same time exclude an effect of the salt-bridge on EGCG activity. In the same study, Superoxide Dismutase (SOD1) was used to prevent EGCG oxidation. Neither modification prevented the remodeling of Aβ fibrils, yet both N-acetylation and Lys-to-Arg mutations prevented the formation of SDS-resistant aggregates. Moreover, treatment with SOD1 resulted in a delayed remodeling of pre-

formed Aβ fibrils into SDS-stable aggregates (Palhano et al. 2013). It should be noted, however, that mutant SOD1 itself is capable of forming amyloid-like fibrils associated with amyotrophic lateral sclerosis (ALS) (Falconi et al. 2013), so that SOD1 might compete with Aβ for EGCG binding. Taken together, these data suggest that covalent cross-linking by EGCG does occur, but is not required for remodeling of Aβ by EGCG.

7.4.4.3 Observations Towards the EGCG Mechanism in IAPP, α-Syn, MSP2 and SEVI

Fewer data are available for EGCG binding to other polypeptides. In IAPP, mutational analysis revealed that site-specific interactions (π-π-stacking, Schiff base formation, disulfide bridge formation) are not necessary for EGCG binding, which implies a non-covalent, non-site-specific binding mode via hydrophobic interactions (Cao and Raleigh 2012). Similarly, when the α-synuclein protein was incubated with EGCG about 30 % of all amino acids lost their NMR-resonances, suggesting an interaction that was not site-specific but likely driven by hydrophobicity (Ehrnhoefer et al. 2008). In an NMR-study with MSP2 only weak interaction of EGCG, mostly with the N-terminal and C-terminal region of the protein could be observed (Chandrashekaran et al. 2010). A pull-down experiment with EGCG-sepharose also found only weak interaction with MSP2; however, interaction might have been sterically hindered, since EGCG was coupled directly to the resin (Chandrashekaran et al. 2010). In contrast, Popovych et al. (2012) found that EGCG interacted with specific residues (amino acids 246–286) of the SEVI peptide, and that prevention of Schiff base formation in SEVI prevented the inhibition of fibrillation.

In conclusion, EGCG seems to interact with polypeptides both through hydrophobic modalities and by site-specific side-chain interactions, most likely through Schiff base formation. Whether covalent interaction or hydrophobic effect drives its anti-amyloid activity seems to depend on the specific polypeptide.

7.5 Conclusion – A Mechanism for Aggregation Redirection by EGCG

A large and growing body of scientific literature demonstrates that EGCG is a potent inhibitor of amyloid formation and amyloid toxicity. Early studies on Htt, α-synuclein and Aβ have identified a mechanism of EGCG that is distinct from other aggregation inhibitors, namely that EGCG redirects amyloidogenic polypeptides into highly stable off-pathway aggregates. Further studies have since characterized a variety of effects of EGCG on different polypeptides. This raises the question whether EGCG acts on amyloidogenic polypeptides by a plethora of mechanisms, or whether these effects may be combined into a unified picture.

Formation of amyloid fibrils proceeds by a common mechanism starting with an amyloidogenic monomer, that can be present under physiological conditions (α-Syn, PrP, Sup35), be released from a precursor as is the case for Aβ and TTR. Amyloidogenicity may result from elevated protein concentrations, as for immunoglobulin light chains and β-microglobin. Aggregation proceeds through a partially or fully unfolded monomeric state (*m*) to partially unstructured oligomer species (*o*) and then to cross-β-sheet oligomer species (*x*), which in the end progress into amyloid fibrils (*f*) (Fig. 7.3b). However, the relative stabilities of these aggregation intermediates result in aggregation kinetics with different rate-limiting steps for each polypeptide.

The variety in the details of the aggregation mechanisms may explain the apparent variety of mechanisms for the action of EGCG. The experimental data suggest that EGCG is indeed interacting with a common binding motif, but that the importance of the EGCG-binding species in the aggregation mechanism may be different depending on the polypeptide.

Data from several polypeptides indicate that EGCG binds to a cross-β-sheet structure, possibly at the same site and in competition with amyloidophilic dyes like ThT. Due to the sterical constraints in the cross-β-sheet structure, the hy-

drophobic side chains of the incorporated amino acids provide a distinct binding surface (Biancalana and Koide 2010). The fact that EGCG is able to remodel preformed fibrils of several amyloidogenic polypeptides implies its ability to bind the common cross-β-sheet structure. In our unified model, binding of EGCG to the cross-β-sheet oligomer (x) results in the species (x_E), which may then be remodeled into an amorphous aggregate (a_E) in case that this species is more stable than x_E (Fig. 7.3b).

Notably, the impact of the binding of EGCG to the amyloid cross-β-sheet motif depends on the energetic aggregation landscape of the specific amyloidogenic polypeptide (Fig. 7.3a). The shape of this landscape depends in turn on the relative stabilities of aggregation competent monomers, aggregation nuclei, oligomeric intermediates and fibrils.

Three modes of aggregation have been observed in studies involving EGCG: (1) the formation of the aggregation-competent species is rate-limiting (e.g. TTR, LC), (2) primary nucleation is rate limiting (e.g. tau), or (3) fibril propagation / secondary nucleation is rate limiting (e.g. Aβ, IAPP). The experimental data for these three mechanisms suggest different energetic states of the EGCG-induced aggregates (x_E) and different positions with respect to the rate-limiting step of aggregation (Fig. 7.3a).

If the formation of a cross-β-sheet nucleus is rate-limiting, then in binding to this rate-limiting species, the effect of EGCG on aggregation kinetics can be profound. The aggregation rate of tau, for instance, depends on the formation of an aggregation nucleus and it is likely that EGCG binds with strong affinity to the cross-β-sheet oligomers x. If EGCG binding remodels the nucleus into a non-amyloid aggregate, EGCG will inhibit aggregation at very low stoichiometric ratios, as is observed in the case of heparin-independent tau aggregation. In Fig. 7.2b, we see that EGCG prevents the large-scale formation of SDS-stable dimers (D), oligomers (O) and high-molecular-weight species (HMW) of tau, likely by sequestering a rate-limiting but non SDS-stable aggregation nucleus, leaving most of the protein in its monomeric state. We could not observe fibril formation under these conditions and therefore were unable to observe if fibril remodeling occurs. Therefore, we cannot be sure whether EGCG redirects tau nuclei into aggregates that have a lower energy than the fibrils.

In contrast, if the cross-β-sheet oligomer is formed after the rate-limiting step, the effect of EGCG on aggregation kinetics is much less potent. Whether EGCG has any effect on aggregation would depend on the relative stabilities of the EGCG-induced aggregate and the amyloid fibril. If the fibril is more stable, then EGCG can still bind to the cross-β-sheet motif, reducing ThT binding, but it will have no effect on aggregation kinetics; such is the case for heparin-induced formation of tau fibrils (Wobst et al. 2015). The same may be true if formation of an aggregation competent species, for example a monomeric amyloid precursor, is rate-limiting. In this case, the rate-limiting species has no cross-β-structure and so EGCG binding does not affect aggregation kinetics. This is likely the case for TTR as well as for LC proteins.

In TTR, the rate-limiting step is the monomerization of the TTR tetramer (Hammarstrom et al. 2003). Under the experimental conditions of LC aggregation that we tested (neutral pH, mildly reducing conditions) the rate-limiting step in aggregation is the monomerization of LC dimers via reduction of disulfide bridges. Without a reduction of the disulfide bridges of light chain dimers, we did not observe aggregation over the course of one month, whereas the reduced protein formed ThT positive aggregates within hours. EGCG can then bind to this aggregate species and accelerate the formation of SDS-stable HMW-species (Fig. 7.2b).

In these cases, even if EGCG did bind to the amyloidogenic precursor, EGCG binding at substoichiometric concentrations cannot deplete its supply and is therefore not able to significantly slow down aggregation kinetics. Instead, the polyphenol seems to accelerate early aggregate formation but inhibit the slower aggregation into fibrillar species, which would be consistent with a nucleation-dependent mechanism for the second step of aggregation, although exact mechanistic details are yet to be explored.

Regarding the Aβ peptide, aggregation into non-β-sheet oligomers can occur rapidly, but nucleus formation is not rate limiting under most experimental conditions. Instead, secondary nucleation processes initiated by fibrillar species dominate the aggregation kinetics (Knowles et al. 2009; Cohen et al. 2013). Under these conditions, EGCG will inhibit aggregation kinetics if it can remodel fibrils into stable aggregates that are not seeding-competent, thereby removing the secondary nuclei. We found that fibril formation of Aβ is quantitatively redirected into SDS-stable amorphous aggregates, which are the most stable species on the aggregation pathway (Ehrnhoefer et al. 2008). Correspondingly, Aβ aggregation in presence of EGCG leads to formation of non-fibrillar aggregates (a_E, Fig. 7.2a). If these are more stable than the fibril, EGCG binding will also result in remodeling of EGCG bound fibrils (f_E) into amorphous aggregates (a_E). In the case of Aβ, EGCG bound to cross-β-sheet species partially disrupts the regular fibril structure and thus promotes the formation of aggregates with only partial β-sheet structure (Bieschke et al. 2010; Lopez del Amo et al. 2012). A similar mechanism may be applicable to IAPP, leading to the remodeling of fibrils into more stable EGCG-induced aggregates.

Our model would predict that the effect of EGCG on aggregation kinetics is lost if the fibrils are more stable than the EGCG-induced aggregates. This was indeed observed for heparin-induced tau fibril formation (Wobst et al. 2015) and may be the case for other amyloidogenic polypeptides for which EGCG would have no effect on aggregation kinetics, such as the NM4 strain of Sup35.

To conclude, the green tea polyphenol EGCG is an intriguing molecule that alters the amyloid aggregation process in novel ways. It has prompted a surprisingly large number of studies that scrutinize its activity on disease-related amyloidogenic proteins and peptides. Its pleiotropic effects on the aggregation cascade of amyloidogenic polypeptides illustrates how subtle differences in the aggregation mechanism may yield very different outcomes of drug intervention. Thus, it remains to be explored whether the mechanistic insight provided by EGCG can be effectively translated into new therapeutic strategies.

References

Abbas S, Wink M (2010) Epigallocatechin gallate inhibits beta amyloid oligomerization in Caenorhabditis elegans and affects the daf-2/insulin-like signaling pathway. Phytomedicine 17(11):902–909. doi:10.1016/j.phymed.2010.03.008

Andrich K, Bieschke J (2014) Aggregation in AL Amyloidosis. Biophys J 106(2):58a. doi:10.1016/j.bpj.2013.11.401

Bae MJ, Ishii T, Minoda K, Kawada Y, Ichikawa T, Mori T, Kamihira M, Nakayama T (2009) Albumin stabilizes (−)-epigallocatechin gallate in human serum: binding capacity and antioxidant property. Mol Nutr Food Res 53(6):709–715. doi:10.1002/mnfr.200800274

Beecher GR (2003) Overview of dietary flavonoids: nomenclature, occurrence and intake. J Nutr 133(10):3248S–3254S

Berson JF, Theos AC, Harper DC, Tenza D, Raposo G, Marks MS (2003) Proprotein convertase cleavage liberates a fibrillogenic fragment of a resident glycoprotein to initiate melanosome biogenesis. J Cell Biol 161(3):521–533. doi:10.1083/jcb.200302072

Biancalana M, Koide S (2010) Molecular mechanism of Thioflavin-T binding to amyloid fibrils. Biochim Biophys Acta 1804(7):1405–1412. doi:10.1016/j.bbapap.2010.04.001

Bieschke J, Russ J, Friedrich RP, Ehrnhoefer DE, Wobst H, Neugebauer K, Wanker EE (2010) EGCG remodels mature alpha-synuclein and amyloid-beta fibrils and reduces cellular toxicity. Proc Natl Acad Sci U S A 107(17):7710–7715. doi:10.1073/pnas.0910723107

Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC (1987) Structure of the human class I histocompatibility antigen, HLA-A2. Nature 329(6139):506–512. doi:10.1038/329506a0

Camilleri A, Zarb C, Caruana M, Ostermeier U, Ghio S, Hogen T, Schmidt F, Giese A, Vassallo N (2013) Mitochondrial membrane permeabilisation by amyloid aggregates and protection by polyphenols. Biochim Biophys Acta 1828(11):2532–2543. doi:10.1016/j.bbamem.2013.06.026

Cao P, Raleigh DP (2012) Analysis of the inhibition and remodeling of islet amyloid polypeptide amyloid fibers by flavanols. Biochemistry 51(13):2670–2683. doi:10.1021/bi2015162

Caruana M, Högen T, Levin J, Hillmer A, Giese A, Vassallo N (2011) Inhibition and disaggregation of α-synuclein oligomers by natural polyphenolic compounds. FEBS Lett 585(8):1113–1120. doi:10.1016/j.febslet.2011.03.046

Chan P (2007) Efficacy and safety of green tea polyphenol in de novo Parkinson's disease patients. http://clinicaltrials.gov/show/NCT00461942

Chandrashekaran IR, Adda CG, MacRaild CA, Anders RF, Norton RS (2010) Inhibition by flavonoids of amyloid-like fibril formation by Plasmodium falciparum merozoite surface protein 2. Biochemistry 49(28):5899–5908. doi:10.1021/bi902197x

Chandrashekaran IR, Adda CG, Macraild CA, Anders RF, Norton RS (2011) EGCG disaggregates amyloid-like fibrils formed by Plasmodium falciparum merozoite surface protein 2. Arch Biochem Biophys 513(2):153–157. doi:10.1016/j.abb.2011.07.008

Chapman MR, Robinson LS, Pinkner JS, Roth R, Heuser J, Hammar M, Normark S, Hultgren SJ (2002) Role of Escherichia coli curli operons in directing amyloid fiber formation. Science 295(5556):851–855. doi:10.1126/science.1067484

Chiti F, Dobson CM (2006) Protein misfolding, functional amyloid, and human disease. Annu Rev Biochem 75:333–366. doi:10.1146/annurev.biochem.75.101304.123901

Cohen SI, Linse S, Luheshi LM, Hellstrand E, White DA, Rajah L, Otzen DE, Vendruscolo M, Dobson CM, Knowles TP (2013) Proliferation of amyloid-beta42 aggregates occurs through a secondary nucleation mechanism. Proc Natl Acad Sci U S A 110(24):9758–9763. doi:10.1073/pnas.1218402110

Colletier JP, Laganowsky A, Landau M, Zhao M, Soriaga AB, Goldschmidt L, Flot D, Cascio D, Sawaya MR, Eisenberg D (2011) Molecular basis for amyloid-beta polymorphism. Proc Natl Acad Sci U S A 108(41):16938–16943. doi:10.1073/pnas.1112600108

Coustou V, Deleu C, Saupe S, Begueret J (1997) The protein product of the het-s heterokaryon incompatibility gene of the fungus Podospora anserina behaves as a prion analog. Proc Natl Acad Sci U S A 94(18):9773–9778

Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, Lin JK, Farzadfar F, Khang YH, Stevens GA, Rao M, Ali MK, Riley LM, Robinson CA, Ezzati M, Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating G (2011) National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. Lancet 378(9785):31–40. doi:10.1016/S0140-6736(11)60679-X

Dedmon MM, Lindorff-Larsen K, Christodoulou J, Vendruscolo M, Dobson CM (2005) Mapping long-range interactions in alpha-synuclein using spin-label NMR and ensemble molecular dynamics simulations. J Am Chem Soc 127(2):476–477. doi:10.1021/ja044834j

Dragicevic N, Smith A, Lin X, Yuan F, Copes N, Delic V, Tan J, Cao C, Shytle RD, Bradshaw PC (2011) Green tea epigallocatechin-3-gallate (EGCG) and other flavonoids reduce Alzheimer's amyloid-induced mitochondrial dysfunction. J Alzheimers Dis 26(3):507–521. doi:10.3233/jad-2011-101629

Edmundson AB, Harris DL, Fan ZC, Guddat LW, Schley BT, Hanson BL, Tribbick G, Geysen HM (1993) Principles and pitfalls in designing site-directed peptide ligands. Proteins 16(3):246–267. doi:10.1002/prot.340160304

Ehrnhoefer DE, Duennwald M, Markovic P, Wacker JL, Engemann S, Roark M, Legleiter J, Marsh JL, Thompson LM, Lindquist S, Muchowski PJ, Wanker EE (2006) Green tea (−)-epigallocatechin-gallate modulates early events in huntingtin misfolding and reduces toxicity in Huntington's disease models. Hum Mol Genet 15(18):2743–2751. doi:10.1093/hmg/ddl210

Ehrnhoefer DE, Bieschke J, Boeddrich A, Herbst M, Masino L, Lurz R, Engemann S, Pastore A, Wanker EE (2008) EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers. Nat Struct Mol Biol 15(6):558–566. doi:10.1038/nsmb.1437

Falconi M, Iacovelli F, Desideri A (2013) A structural modeling approach for the understanding of initiation and elongation of ALS-linked superoxide dismutase fibrils. J Mol Model 19(9):3695–3704. doi:10.1007/s00894-013-1896-7

Ferreira N, Saraiva MJ, Almeida MR (2011) Natural polyphenols inhibit different steps of the process of transthyretin (TTR) amyloid fibril formation. FEBS Lett 585(15):2424–2430. doi:10.1016/j.febslet.2011.06.030

Ferreira N, Saraiva MJ, Almeida MR (2012a) Epigallocatechin-3-gallate as a potential therapeutic drug for TTR-related amyloidosis: "in vivo" evidence from FAP mice models. PLoS One 7(1), e29933. doi:10.1371/journal.pone.0029933

Ferreira N, Saraiva MJ, Almeida MR (2012b) Natural polyphenols as modulators of TTR amyloidogenesis: in vitro and in vivo evidences towards therapy. Amyloid 19(Suppl 1):39–42. doi:10.3109/13506129.2012.668502

Flach K, Hilbrich I, Schiffmann A, Gartner U, Kruger M, Leonhardt M, Waschipky H, Wick L, Arendt T, Holzer M (2012) Tau oligomers impair artificial membrane integrity and cellular viability. J Biol Chem 287(52):43223–43233. doi:10.1074/jbc.M112.396176

Fowler DM, Koulov AV, Alory-Jost C, Marks MS, Balch WE, Kelly JW (2006) Functional amyloid formation within mammalian tissue. PLoS Biol 4(1):e6. doi:10.1371/journal.pbio.0040006

Friedemann P, Dörr J (2009) Sunphenon EGCg (Epigallocatechin-Gallate) in the early stage of Alzheimer's disease (SUN-AK). 2009 edn. U.S. National Institutes of Health. http://clinicaltrials.gov/show/NCT00951834

Gauci AJ, Caruana M, Giese A, Scerri C, Vassallo N (2011) Identification of polyphenolic compounds and black tea extract as potent inhibitors of lipid membrane destabilization by Abeta42 aggregates. J Alzheimers Dis 27(4):767–779. doi:10.3233/JAD-2011-111061

Genton B, Betuela I, Felger I, Al-Yaman F, Anders RF, Saul A, Rare L, Baisor M, Lorry K, Brown GV, Pye D, Irving DO, Smith TA, Beck HP, Alpers MP (2002)

A recombinant blood-stage malaria vaccine reduces Plasmodium falciparum density and exerts selective pressure on parasite populations in a phase 1-2b trial in Papua New Guinea. J Infect Dis 185(6):820–827. doi:10.1086/339342

Gejyo F, Yamada T, Odani S, Nakagawa Y, Arakawa M, Kunitomo T, Kataoka H, Suzuki M, Hirasawa Y, Shirahama T (1985) A new form of amyloid protein associated with chronic hemodialysis was identified as beta 2-microglobulin. Biochem Biophys Res Commun 129(3):701–706. doi:10.1016/0006-291X(85)91948-5

Giunta B, Hou H, Zhu Y, Salemi J, Ruscin A, Shytle RD, Tan J (2010) Fish oil enhances anti-amyloidogenic properties of green tea EGCG in Tg2576 mice. Neurosci Lett 471(3):134–138. doi:10.1016/j.neulet.2010.01.026

Golde TE, Cai XD, Shoji M, Younkin SG (1993) Production of amyloid beta protein from normal amyloid beta-protein precursor (beta APP) and the mutated beta APPS linked to familial Alzheimer's disease. Ann N Y Acad Sci 695:103–108

Graham HN (1992) Green tea composition, consumption, and polyphenol chemistry. Prev Med 21(3):334–350

Grelle G, Otto A, Lorenz M, Frank RF, Wanker EE, Bieschke J (2011) Black tea theaflavins inhibit formation of toxic amyloid-beta and alpha-synuclein fibrils. Biochemistry 50(49):10624–10636. doi:10.1021/bi2012383

Grey HM, Kubo RT, Colon SM, Poulik MD, Cresswell P, Springer T, Turner M, Strominger JL (1973) The small subunit of HL-A antigens is beta 2-microglobulin. J Exp Med 138(6):1608–1612

Haass C, Selkoe DJ (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat Rev Mol Cell Biol 8(2):101–112. doi:10.1038/nrm2101

Hammarstrom P, Wiseman RL, Powers ET, Kelly JW (2003) Prevention of transthyretin amyloid disease by changing protein misfolding energetics. Science 299(5607):713–716. doi:10.1126/science.1079589

Hauber I, Hohenberg H, Holstermann B, Hunstein W, Hauber J (2009) The main green tea polyphenol epigallocatechin-3-gallate counteracts semen-mediated enhancement of HIV infection. Proc Natl Acad Sci U S A 106(22):9033–9038. doi:10.1073/pnas.0811827106

He Y, Cui J, Lee JC, Ding S, Chalimoniuk M, Simonyi A, Sun AY, Gu Z, Weisman GA, Wood WG, Sun GY (2011) Prolonged exposure of cortical neurons to oligomeric amyloid-beta impairs NMDA receptor function via NADPH oxidase-mediated ROS production: protective effect of green tea (−)-epigallocatechin-3-gallate. ASN Neuro 3(1), e00050. doi:10.1042/an20100025

Hunstein W (2007) Epigallocathechin-3-gallate in AL amyloidosis: a new therapeutic option? Blood 110(6):2216. doi:10.1182/blood-2007-05-089243

Ishii T, Ichikawa T, Minoda K, Kusaka K, Ito S, Suzuki Y, Akagawa M, Mochizuki K, Goda T, Nakayama T (2011) Human serum albumin as an antioxidant in the oxidation of (−)-epigallocatechin gallate: participation of reversible covalent binding for interaction and stabilization. Biosci Biotechnol Biochem 75(1):100–106. doi:10.1271/bbb.100600

Jarrett JT, Lansbury PT Jr (1993) Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? Cell 73(6):1055–1058

Johnson KH, O'Brien TD, Betsholtz C, Westermark P (1989) Islet amyloid, islet-amyloid polypeptide, and diabetes mellitus. N Engl J Med 321(8):513–518. doi:10.1056/NEJM198908243210806

Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 300(5618):486–489. doi:10.1126/science.1079469

Knowles TP, Waudby CA, Devlin GL, Cohen SI, Aguzzi A, Vendruscolo M, Terentjev EM, Welland ME, Dobson CM (2009) An analytical solution to the kinetics of breakable filament assembly. Science 326(5959):1533–1537. doi:10.1126/science.1178250

Kosik KS, Joachim CL, Selkoe DJ (1986) Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. Proc Natl Acad Sci U S A 83(11):4044–4048

Kristen AV, Lehrke S, Buss S, Mereles D, Steen H, Ehlermann P, Hardt S, Giannitsis E, Schreiner R, Haberkorn U, Schnabel PA, Linke RP, Rocken C, Wanker EE, Dengler TJ, Altland K, Katus HA (2012) Green tea halts progression of cardiac transthyretin amyloidosis: an observational report. Clin Res Cardiol 101(10):805–813. doi:10.1007/s00392-012-0463-z

Lambert JD, Sang S, Yang CS (2008) N-Acetylcysteine enhances the lung cancer inhibitory effect of epigallocatechin-3-gallate and forms a new adduct. Free Radic Biol Med 44(6):1069–1074. doi:10.1016/j.freeradbiomed.2007.12.016

Lee JW, Lee YK, Ban JO, Ha TY, Yun YP, Han SB, Oh KW, Hong JT (2009a) Green tea (−)-epigallocatechin-3-gallate inhibits beta-amyloid-induced cognitive dysfunction through modification of secretase activity via inhibition of ERK and NF-kappaB pathways in mice. J Nutr 139(10):1987–1993. doi:10.3945/jn.109.109785

Lee YK, Yuk DY, Lee JW, Lee SY, Ha TY, Oh KW, Yun YP, Hong JT (2009b) (−)-Epigallocatechin-3-gallate prevents lipopolysaccharide-induced elevation of beta-amyloid generation and memory deficiency. Brain Res 1250:164–174. doi:10.1016/j.brainres.2008.10.012

Lee YJ, Choi DY, Yun YP, Han SB, Oh KW, Hong JT (2013) Epigallocatechin-3-gallate prevents systemic inflammation-induced memory deficiency and amyloidogenesis via its anti-neuroinflammatory properties. J Nutr Biochem 24(1):298–310. doi:10.1016/j.jnutbio.2012.06.011

LeVine H 3rd (1997) Stopped-flow kinetics reveal multiple phases of thioflavin T binding to Alzheimer beta (1–40) amyloid fibrils. Arch Biochem Biophys 342(2):306–316. doi:10.1006/abbi.1997.0137

LeVine H 3rd (1999) Quantification of beta-sheet amyloid fibril structures with thioflavin T. Methods Enzymol 309:274–284

Levites Y, Amit T, Mandel S, Youdim MB (2003) Neuroprotection and neurorescue against Abeta toxicity and PKC-dependent release of nonamyloidogenic soluble precursor protein by green tea polyphenol (−)-epigallocatechin-3-gallate. FASEB J 17(8):952–954. doi:10.1096/fj.02-0881fje

Li X, Zhang X, Ladiwala AR, Du D, Yadav JK, Tessier PM, Wright PE, Kelly JW, Buxbaum JN (2013) Mechanisms of transthyretin inhibition of beta-amyloid aggregation in vitro. J Neurosci 33(50):19423–19433. doi:10.1523/JNEUROSCI.2561-13.2013

Lin CL, Chen TF, Chiu MJ, Way TD, Lin JK (2009) Epigallocatechin gallate (EGCG) suppresses beta-amyloid-induced neurotoxicity through inhibiting c-Abl/FE65 nuclear translocation and GSK3 beta activation. Neurobiol Aging 30(1):81–92. doi:10.1016/j.neurobiolaging.2007.05.012

Linke RP (1985) Immunochemical typing of amyloid deposits after microextraction from biopsies. Appl Pathol 3(1–2):18–28

Lopez del Amo JM, Fink U, Dasari M, Grelle G, Wanker EE, Bieschke J, Reif B (2012) Structural properties of EGCG-induced, nontoxic Alzheimer's disease Abeta oligomers. J Mol Biol 421(4–5):517–524. doi:10.1016/j.jmb.2012.01.013

Lorenzen N, Nielsen SB, Yoshimura Y, Vad BS, Andersen CB, Betzer C, Kaspersen JD, Christiansen G, Pedersen JS, Jensen PH, Mulder FA, Otzen DE (2014) How epigallocatechin gallate can inhibit alpha-synuclein oligomer toxicity in vitro. J Biol Chem. doi:10.1074/jbc.M114.554667

Lu JX, Qiang W, Yau WM, Schwieters CD, Meredith SC, Tycko R (2013) Molecular structure of beta-amyloid fibrils in Alzheimer's disease brain tissue. Cell 154(6):1257–1268. doi:10.1016/j.cell.2013.08.035

Luhrs T, Ritter C, Adrian M, Riek-Loher D, Bohrmann B, Dobeli H, Schubert D, Riek R (2005) 3D structure of Alzheimer's amyloid-beta(1–42) fibrils. Proc Natl Acad Sci U S A 102(48):17342–17347. doi:10.1073/pnas.0506723102

Maiti TK, Ghosh KS, Dasgupta S (2006) Interaction of (−)-epigallocatechin-3-gallate with human serum albumin: fluorescence, fourier transform infrared, circular dichroism, and docking studies. Proteins 64(2):355–362. doi:10.1002/prot.20995

Meng F, Abedini A, Plesner A, Verchere CB, Raleigh DP (2010) The flavanol (−)-epigallocatechin 3-gallate inhibits amyloid formation by islet amyloid polypeptide, disaggregates amyloid fibrils, and protects cultured cells against IAPP-induced toxicity. Biochemistry 49(37):8127–8133. doi:10.1021/bi100939a

Mereles D, Wanker EE, Katus HA (2008) Therapy effects of green tea in a patient with systemic light-chain amyloidosis. Clin Res Cardiol 97(5):341–344. doi:10.1007/s00392-008-0649-6

Mereles D, Buss SJ, Hardt SE, Hunstein W, Katus HA (2010) Effects of the main green tea polyphenol epigallocatechin-3-gallate on cardiac involvement in patients with AL amyloidosis. Clin Res Cardiol 99(8):483–490. doi:10.1007/s00392-010-0142-x

Merlini GPG (2013) Epigallocatechingallate (EGCG) in cardiac AL amyloidosis (EpiCardiAL). http://www.clinicaltrial.gov/ct2/show/record/NCT01511263

Miyata M, Sato T, Kugimiya M, Sho M, Nakamura T, Ikemizu S, Chirifu M, Mizuguchi M, Nabeshima Y, Suwa Y, Morioka H, Arimori T, Suico MA, Shuto T, Sako Y, Momohara M, Koga T, Morino-Koga S, Yamagata Y, Kai H (2010) The crystal structure of the green tea polyphenol (−)-epigallocatechin gallate-transthyretin complex reveals a novel binding site distinct from the thyroxine binding site. Biochemistry 49(29):6104–6114. doi:10.1021/bi1004409

Nozaki A, Hori M, Kimura T, Ito H, Hatano T (2009) Interaction of polyphenols with proteins: binding of (−)-epigallocatechin gallate to serum albumin, estimated by induced circular dichroism. Chem Pharm Bull 57(2):224–228

Palhano FL, Lee J, Grimster NP, Kelly JW (2013) Toward the molecular mechanism(s) by which EGCG treatment remodels mature amyloid fibrils. J Am Chem Soc 135(20):7503–7510. doi:10.1021/ja3115696

Paz MA, Fluckiger R, Boak A, Kagan HM, Gallop PM (1991) Specific detection of quinoproteins by redox-cycling staining. J Biol Chem 266(2):689–692

Permutt A, Chirgwin J, Giddings S, Kakita K, Rotwein P (1981) Insulin biosynthesis and diabetes mellitus. Clin Biochem 14(5):230–236

Petkova AT, Yau WM, Tycko R (2006) Experimental constraints on quaternary structure in Alzheimer's beta-amyloid fibrils. Biochemistry 45(2):498–512. doi:10.1021/bi051952q

Popovych N, Brender JR, Soong R, Vivekanandan S, Hartman K, Basrur V, Macdonald PM, Ramamoorthy A (2012) Site specific interaction of the polyphenol EGCG with the SEVI amyloid precursor peptide PAP(248–286). J Phys Chem B 116(11):3650–3658. doi:10.1021/jp2121577

Priller J (2011) Effects of EGCG (Epigallocatechin Gallate) in Huntington's disease (ETON-Study). https://clinicaltrials.gov/ct2/show/NCT01357681

Prince M, Albanese E, Guerchet M, Prina M (2014) World Alzheimer Report 2014 – Dementia and risk reduction – an analysis of protective and modifiable factors. World Alzheimer Report. Alzheimer's Disease International – The global voice on dementia

Prusiner SB (1998) Prions. Proc Natl Acad Sci U S A 95(23):13363–13383. doi:10.1073/pnas.95.23.13363

Rambold AS, Miesbauer M, Olschewski D, Seidel R, Riemer C, Smale L, Brumm L, Levy M, Gazit E, Oesterhelt D, Baier M, Becker CF, Engelhard M, Winklhofer KF, Tatzelt J (2008) Green tea extracts interfere with the stress-protective activity of PrP and the formation of PrP. J Neurochem 107(1):218–229. doi:10.1111/j.1471-4159.2008.05611.x

Redegeld FA, Nijkamp FP (2003) Immunoglobulin free light chains and mast cells: pivotal role in T-cell-mediated immune reactions? Trends Immunol 24(4):181–185

Rezai-Zadeh K, Shytle D, Sun N, Mori T, Hou H, Jeanniton D, Ehrhart J, Townsend K, Zeng J, Morgan D, Hardy J, Town T, Tan J (2005) Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. J Neurosci 25(38):8807–8814. doi:10.1523/jneurosci.1521-05.2005

Rezai-Zadeh K, Arendash GW, Hou H, Fernandez F, Jensen M, Runfeldt M, Shytle RD, Tan J (2008) Green tea epigallocatechin-3-gallate (EGCG) reduces beta-amyloid mediated cognitive impairment and modulates tau pathology in Alzheimer transgenic mice. Brain Res 1214:177–187. doi:10.1016/j.brainres.2008.02.107

Ribeiro CA, Saraiva MJ, Cardoso I (2012) Stability of the transthyretin molecule as a key factor in the interaction with A-beta peptide–relevance in Alzheimer's disease. PLoS One 7(9), e45368. doi:10.1371/journal.pone.0045368

Riek R, Hornemann S, Wider G, Billeter M, Glockshuber R, Wuthrich K (1996) NMR structure of the mouse prion protein domain PrP(121–231). Nature 382(6587):180–182. doi:10.1038/382180a0

Roberts BE, Duennwald ML, Wang H, Chung C, Lopreiato NP, Sweeny EA, Knight MN, Shorter J (2009) A synergistic small-molecule combination directly eradicates diverse prion strain structures. Nat Chem Biol 5(12):936–946. doi:10.1038/nchembio.246

Rodrigues CM, Sola S, Silva R, Brites D (2000) Bilirubin and amyloid-beta peptide induce cytochrome c release through mitochondrial membrane permeabilization. Mol Med 6(11):936–946

Saraiva MJ, Birken S, Costa PP, Goodman DS (1984) Family studies of the genetic abnormality in transthyretin (prealbumin) in Portuguese patients with familial amyloidotic polyneuropathy. Ann N Y Acad Sci 435:86–100

Scheraga HA, Nemethy G, Steinberg IZ (1962) The contribution of hydrophobic bonds to the thermal stability of protein conformations. J Biol Chem 237:2506–2508

Scherzinger E, Lurz R, Turmaine M, Mangiarini L, Hollenbach B, Hasenbank R, Bates GP, Davies SW, Lehrach H, Wanker EE (1997) Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates in vitro and in vivo. Cell 90(3):549–558. doi:10.1016/s0092-8674(00)80514-0

Schönland SO (2013) A trial for the treatment of cardiac AL-Amyloidosis with the green tea compound epigallocatechin-3-gallate (TAME-AL). http://www.clinicaltrial.gov/ct2/show/record/NCT02015312

Shorter J, Lindquist S (2004) Hsp104 catalyzes formation and elimination of self-replicating Sup35 prion conformers. Science 304(5678):1793–1797. doi:10.1126/science.1098007

Shorter J, Lindquist S (2005) Prions as adaptive conduits of memory and inheritance. Nat Rev Genet 6(6):435–450. doi:10.1038/nrg1616

Sinha S, Du Z, Maiti P, Klarner FG, Schrader T, Wang C, Bitan G (2012) Comparison of three amyloid assembly inhibitors: the sugar scyllo-inositol, the polyphenol epigallocatechin gallate, and the molecular tweezer CLR01. ACS Chem Neurosci 3(6):451–458. doi:10.1021/cn200133x

Solomon A, Frangione B, Franklin EC (1982) Bence Jones proteins and light chains of immunoglobulins. Preferential association of the V lambda VI subgroup of human light chains with amyloidosis AL (lambda). J Clin Invest 70(2):453–460

Suzuki Y, Brender JR, Hartman K, Ramamoorthy A, Marsh EN (2012) Alternative pathways of human islet amyloid polypeptide aggregation distinguished by (19)f nuclear magnetic resonance-detected kinetics of monomer consumption. Biochemistry 51(41):8154–8162. doi:10.1021/bi3012548

Takeda A, Mallory M, Sundsmo M, Honer W, Hansen L, Masliah E (1998) Abnormal accumulation of NACP/alpha-synuclein in neurodegenerative disorders. Am J Pathol 152(2):367–372

Trottier Y, Devys D, Imbert G, Saudou F, An I, Lutz Y, Weber C, Agid Y, Hirsch EC, Mandel JL (1995) Cellular localization of the Huntington's disease protein and discrimination of the normal and mutated form. Nat Genet 10(1):104–110. doi:10.1038/ng0595-104

Ulmer TS, Bax A, Cole NB, Nussbaum RL (2005) Structure and dynamics of micelle-bound human alpha-synuclein. J Biol Chem 280(10):9595–9603. doi:10.1074/jbc.M411805200

Volles MJ, Lee SJ, Rochet JC, Shtilerman MD, Ding TT, Kessler JC, Lansbury PT Jr (2001) Vesicle permeabilization by protofibrillar alpha-synuclein: implications for the pathogenesis and treatment of Parkinson's disease. Biochemistry 40(26):7812–7819

Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ (2002a) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature 416(6880):535–539. doi:10.1038/416535a

Walsh DM, Klyubin I, Fadeeva JV, Rowan MJ, Selkoe DJ (2002b) Amyloid-beta oligomers: their production, toxicity and therapeutic inhibition. Biochem Soc Trans 30(4):552–557. doi: 10.1042/bst0300552

Wang SH, Liu FF, Dong XY, Sun Y (2010) Thermodynamic analysis of the molecular interactions between amyloid beta-peptide 42 and (−)-epigallocatechin-3-gallate. J Phys Chem B 114(35):11576–11583. doi:10.1021/jp1001435

Wang SH, Dong XY, Sun Y (2012a) Effect of (−)-epigallocatechin-3-gallate on human insulin fibrillation/aggregation kinetics. Biochem Eng J 63:38–49. doi:10.1016/j.bej.2012.02.002

Wang SH, Dong XY, Sun Y (2012b) Thermodynamic analysis of the molecular interactions between amyloid beta-protein fragments and (−)-epigallocatechin-3-gallate. J Phys Chem B 116(20):5803–5809. doi:10.1021/jp209406t

Wanker EE, Scherzinger E, Heiser V, Sittler A, Eickhoff H, Lehrach H (1999) Membrane filter assay for detection of amyloid-like polyglutamine-containing protein aggregates. Methods Enzymol 309: 375–386

Wobst HJ, Sharma A, Diamond MI, Wanker EE, Bieschke J (2015) The green tea polyphenol (−)-epigallocatechin gallate prevents the aggregation of tau protein into toxic oligomers at substoichiometric ratios. FEBS Lett. 589(1):77–83. doi:10.1016/j.febslet.2014.11.026

Wojtczak A, Luft J, Cody V (1992) Mechanism of molecular recognition. Structural aspects of 3,3′-diiodo-L-thyronine binding to human serum transthyretin. J Biol Chem 267(1):353–357

Woods LA, Platt GW, Hellewell AL, Hewitt EW, Homans SW, Ashcroft AE, Radford SE (2011) Ligand binding to distinct states diverts aggregation of an amyloid-forming protein. Nat Chem Biol 7(10):730–739. doi:10.1038/nchembio.635

Yang X, Adda CG, Keizer DW, Murphy VJ, Rizkalla MM, Perugini MA, Jackson DC, Anders RF, Norton RS (2007) A partially structured region of a largely unstructured protein, Plasmodium falciparum merozoite surface protein 2 (MSP2), forms amyloid-like fibrils. J Pept Sci 13(12):839–848. doi:10.1002/psc.910

Young LM, Cao P, Raleigh DP, Ashcroft AE, Radford SE (2014) Ion mobility spectrometry-mass spectrometry defines the oligomeric intermediates in amylin amyloid formation and the mode of action of inhibitors. J Am Chem Soc 136(2):660–670. doi:10.1021/ja406831n

Zhang T, Faraggi E, Li Z, Zhou Y (2013) Intrinsically semi-disordered state and its role in induced folding and protein aggregation. Cell Biochem Biophys 67(3):1193–1205. doi:10.1007/s12013-013-9638-0

Index

N. Vassallo (ed.), *Natural Compounds as Therapeutic Agents for Amyloidogenic Diseases*,
Advances in Experimental Medicine and Biology 863, DOI 10.1007/978-3-319-18365-7