Acta Neuropathol (2006) 111: 503–509
DOI 10.1007/s00401-006-0071-y

REVIEW

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Neuropathology of Alzheimer disease: pathognomonic but not pathogenic

Received: 28 June 2005 / Revised: 29 March 2006 / Accepted: 29 March 2006 / Published online: 27 April 2006

Abstract Neuropathological changes in subjects with dementia are, by definition, end-stage phenomena. While such changes allow case characterization and lend themselves to disease classification and modeling, the lesions themselves are not etiological. This truth would appear to be self-evident, yet the medical and scientific literature suggests otherwise. Indeed it is now customary to view amyloid plaques in Alzheimer disease as primary etiological, neurotoxic lesions and, hence, removing them (e.g., by immunotherapy) is believed to lead to clinical improvement. The foundation for this line of thinking lies in the existence of rare kindreds with mutations in amyloid-β, or mutations believed to be involved in the processing of amyloid-β, and then the extrapolation of the inherited condition to sporadic disease. We believe that this overall construct ignores early events that are more critical to onset and progression of sporadic disease. Likewise, we have studied subjects with sporadic Alzheimer disease, as well as early onset familial Alzheimer disease and Down's syndrome, over a spectrum of ages, and have found that markers of oxidative stress precede amyloid deposits in all three conditions. Amyloid and neurofibrillary pathology in the Alzheimer brain show a decrease in oxidative stress relative to vulnerable but morphologically intact neurons, suggesting that neurodegenerative lesions are compensatory phenomena, and thus manifestations of cellular adaptation. The pathology of neurodegenerative diseases should be viewed as the end-stage consequence, as opposed to cause, of the disease processes, so that early disease processes that are amenable to intervention can be properly recognized and treated.

Keywords Alzheimer disease · Amyloid · Neuropathology · Tau phosphorylation

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Introduction

Neuropathological assessment by light microscopy is regarded as a means of definitive diagnosis of patients with dementia, and further establishes benchmarks by which models of neurodegenerative diseases are validated. This being the case, it is axiomatic that the basic pathology of dementia is over-rated in terms of insight into early disease processes.

The simple fact, which is not new but rather ignored, is that neuropathological diagnosis, in the setting of neurodegenerative diseases, is little more than a "tallying" of lesions at the end of life, be they plaques, tangles, or other inclusion; the key to proper diagnosis rests more in the association of that tally with a clinical phenotype during life than in the identification of a pathogenic process [28, 38]. Indeed, disease etiology, within the context of dementia brain interpretation, is irrelevant.

Yet we are fascinated with lesions—amyloid plaques, neurofibrillary tangles, Lewy bodies, etc. and study them extensively using every conceivable modality. This is not particularly surprising from the standpoint of the neuropathologist, since we rely on those changes that can be visualized and, without them, we are rendered impotent in our ability to assess disease. Nevertheless, the perseveration on lesions, not only by neuropathologists but

also across the spectrum of neuroscience disciplines, in the form of thousands upon thousands of research articles and millions upon millions of tax dollars, clearly demonstrates a presumption, whether we admit it or not, that those lesions hold great value, greater perhaps than the facts warrant, and that the key to etiology lies within them. Such gun barrel vision may be myopic in the larger picture of a complex life-long, non-neoplastic process whose etiology remains to be defined, and may also be dubious in light of today's rush to "translate" bench data to living patients. In this review, we overview pathological lesions in AD and emphasize the notion that microscopy says more about effect than cause. With some necessary reorganization of thinking, we hope to provide impetus for more innovated approaches to studies and therapies.

Plaques, amyloid-β, and a hypothesis

Senile plaques were recognized as early as Alzheimer's original case, described as "miliary foci" of peculiar material on Bielschowsky silver preparations [2]. At that time, miliary foci were as much qualitative as quantitative, being a novel finding in the brain of a patient with a neuropsychiatric condition, although it has since become clear that senile plaques are often present in significant numbers in the brains of cognitively intact elderly individuals [23, 65]. Subsequent identification of the histochemical properties of amyloid within plaque cores, and then amyloid-β as the major protein component, understandably raised the possibility of a protein-mediated neurotoxic process, and laid the foundation for a hypothesis that has changed only marginally in the past 20 years.

The perceived strength in the amyloid cascade hypothesis is reflected in the scientific literature, which is voluminous and dominated by experimental studies that strictly adhere to the hypothesis [26]. The human data, however, is more critical to the validity of the hypothesis and centers around the following: (1) amyloid-β accumulates in senile plaques in the AD brain; (2) specific point mutations in the gene for AβPP cause familial, early onset AD; and (3) increased copy numbers of AβPP in some cases of Down's syndrome lead to relatively early amyloid-β deposits and pathology generally associated with AD. In essence, human studies have identified genetic lesions with an aberrant protein-driven phenotype. Hence the extrapolation that amyloid-β synthesis and deposition, and in particular a relative increase in the synthesis and deposition of "pathogenic" amyloid-β42, must be the "rate-limiting" factor in AD pathogenesis, while accompanying pathology (neurofibrillary pathology, neuronal loss, synaptic dysfunction) are secondary, end-organ phenomena.

The subsequent identification of additional, and now more numerous, kindreds carrying mutations in the presenilins did not hamper the amyloid cascade hypothesis, but rather proposed to substantiate it, as the evidence that presenilins were necessary components of the γ-secretase complex in Notch proteolysis, and by extension AβPP (necessary along with β-secretase for cleavage of AβPP and production of amyloid-β42), quickly appeared in the literature [16, 56, 63, 67]. So not only were there genotype–phenotype correlations, but now a putative enzyme–substrate relationship between the various proteins lesioned in familial early onset AD, and thus a more detailed proposal of a biochemical cascade.

It may be noted that in light of the prodigious accumulations of amyloid-β in presenilin-linked familial AD, and the requirement for the existence of γ-secretase, the relationship between presenilins and AβPP, and indeed the assignment of γ-secretase function to presenilins, was for practical purposes pre-ordained, and the task of scientists given to the amyloid cascade hypothesis became the discovery of data that supported the enzyme-substrate paradigm, rather than determine *whether or not* a relationship existed in the first place. In this vein, it is perhaps not surprising that supporting evidence has been found in abundance, and that it is now second nature to view the presenilins and γ-secretase as the same. This is in spite of the evidence being based on in vivo data from worms, flies, and transgenic mice, and that little is known about the structure of γ-secretase, the mechanism it utilizes for proteolysis, or the regulation of cleavage [7].

Familial cerebral amyloidosis and amyloid-β as a rate-limiting factor

Just as it is hypothesized that amyloid-β42 production is the rate-limiting factor in AD pathogenesis, the validity of an extrapolation of familial early onset AD to sporadic disease is a "rate-limiting" factor for the amyloid cascade hypothesis. In this regard, it is necessary to point out that total identified familial early onset AD kindreds, with known mutations number about 400; of these, AβPP kindreds number less than 100 and PS2 mutation kindreds less than 20 (Alzheimer's Disease and Frontotemporal Dementia Mutation Database, http://www.molgen.ua.ac.be/ADMutations/default.cfm). This is in contrast to the denominator of dementia subjects that number at least 20 million across the globe. Thus the genotype–phenotype relationship, or the genotype–enzyme/substrate relationship, lacks the genotype portion of the equation in the vast majority of AD subjects, while other risk factors (Apolipoprotein E polymorphism, head trauma, diet, sex hormones, educational background, aluminum exposure, etc.) come into play, many of which are either unaccounted for by the amyloid cascade hypothesis, or accounted for only on an ad hoc basis. On the other hand, the phenotype portion of the equation in terms of neuropathology, and in terms of clinical disease, indicates that end-organ damage is heterogeneous, both within the early onset familial AD group and relative to sporadic AD. Clinically, presentations that include cerebral hemorrhage, spastic paraparesis

with delayed dementia, and subcortical dementia with Parkinsonism [12, 29, 55] clearly differ from sporadic AD, while pathologically the extensive amyloid burden including extensive white matter, deep gray matter, and cerebellar amyloid, and "cotton wool" plaques that lack fibrillar amyloid in presenilin 1 mutation cases also differ from classical sporadic AD. These clinicopathological data suggest overall that early onset familial AD imperfectly mimics the far more common sporadic condition. While "early-onset familial AD" is a term embedded in the literature, "early onset cerebral amyloidosis" is perhaps a more objective term to describe this small group of Mendelian conditions.

One might also look to other neurodegenerative and other processes with the question of sporadic disease extrapolation from autosomal dominant disease. For example, is Cu–Zn superoxide dismutase alteration the rate-limiting factor for sporadic amyotrophic lateral sclerosis (ALS) because a small fraction of ALS subjects carry a germline mutation in superoxide dismutase? Is α-synuclein alteration the rate-limiting factor for sporadic Parkinson's disease (PD) because rare PD kindreds carry a germline mutation in α-synuclein? Is p53 protein alteration the rate-limiting factor for the development of sporadic glioblastoma multiforme because patients with Li-Fraumeni syndrome and germline TP53 mutation are predisposed to glioblastoma multiforme? Is LDL cholesterol alteration the rate-limiting factor for sporadic atherosclerotic cardiovascular disease because patients with familial autosomal dominant hypercholesteremia consistently development atherosclerotic cardiovascular disease at a young age? In each instance, as in AD, the rare genetic syndromes are useful, but they are not the beginning and the end of the pathogenesis overall. Rather, sporadic disease is multifactorial and modeled only imperfectly by rare kindreds with strict Mendelian genetic aberrations.

Oxidative stress and amyloid

The overlap between AD pathology and brain changes in cognitively intact elderly individuals, and the overall poor correlation between amyloid deposits and clinical signs, is reflected in standard criteria that use terms such "probable," "possible," "high likelihood," "intermediate likelihood," etc. [1, 38]. Statistically, the more lenient the criteria (i.e., the more sensitive the criteria), the less specific the neuropathology, and the greater the number of subjects will be inaccurately assigned to the AD category. Clearly, therefore, the amyloid plaque is not "diagnostic" of anything, but is rather a marker of disease with certain sensitivities and specificities based on quantity and the association with clinical signs.

This problem is due in part to the fact that neuropathology assesses, by definition, the end point. Far more useful in terms of pathogenesis are those changes that occur early in disease, i.e., preclinically or within neurons unaffected by inclusion formation. In this respect, the oxidative stress cascade assumes greater significance. We have found that oxidative stress as measured by several endpoints such as 8-hydroxyguanosine (8OHG) and nitrotyrosine adduct formation, precedes amyloid-β deposition by decades in Down's syndrome, sporadic AD, and familial AD [40–44]. Moreover, in brains with AD pathology, lesional tissue (plaques and tangles), were associated with a *decrease* in oxidative stress markers compared to histologically unaffected but vulnerable neurons. Similarly, in Down's syndrome, 8OHG immunoreactivity increased significantly in the teens and twenties, while amyloid-β burden only increased after age 30. In nine cases of Down's syndrome bearing amyloid-β deposition, the extent of amyloid-β42 deposits was actually associated with a decrease in relative 8OHG while amyloid-β40 was not. These data are further evidence that amyloid plaques are likely a compensatory change to the fundamental biochemical cascade that is precipitated by age-associated oxidative stress. Amyloid plaques are thus a "marker" of disease with borderline diagnostic value, and a consequence of the pathophysiology rather than a cause.

It is now known that neurons respond to oxidative stress by increasing amyloid-β production [66] and that this increased amyloid-β is associated with a consequent reduction in oxidative stress [41, 42]. Similarly, we recently demonstrated that amyloid-β is a genuine antioxidant that can act as a potent superoxide dismutase [14]. By this logic, therefore, AD kindreds with AβPP mutations lose effective anti-oxidant capacity (due to mutation-driven protein dysfunction), while the extensive amyloid-β deposits themselves are signatures not of neurotoxicity per se but of oxidative imbalance and an oxidative stress response. This is consistent with the data that amyloid-β deposits begin to appear around age 40 [42], and manifestly more logical than the alternative view that everyone at mid-life is on the verge of developing AD, a view also directly contradicted by the fact that a large percentage of cognitively-intact, aged individuals contain amyloid-β loads equivalent to patients with AD [15].

Fibrillar or aggregated forms of amyloid-β, such as in senile plaque cores, in the obviously artificial cell culture environment are toxic to cultured neurons in vitro [47, 49]. However, in vivo, the presence and density of amyloid-β correlates weakly with the onset and severity of AD [5, 22], while recent data suggests that the presence of the soluble form of amyloid-β in the brain may be a better predictor of the disease [37, 57]. Specifically, SDS-stable oligomers, and not monomers, of this form of amyloid-β seem to play an important role, as shown by augmented presence of these oligomers during the expression of mutations in AβPP or presenilin [64], as well as by their capacity to inhibit neuronal plasticity parameters (LTP) in vivo when micro-injected into the brains of rodents [60].

Conversely, amyloid-β deposits are not always present in the brains of cognitively normal elderly. Whether this indicates that some individuals have efficient endog-

enous antioxidant defense systems and thus age more effectively, or whether such individuals may have supplemented their diets with antioxidants throughout their lifespan, compensating for age-related declines in antioxidant defenses, remains to be elucidated [6, 31, 32]. If amyloid-β deposition possesses antioxidant function, this process will be recruited during times when oxidative stress is high and the endogenous antioxidant-defenses are compromised. On the other hand, if this system is efficient and/or is supported by exogenous antioxidant supplementation, the anti-oxidant effects of amyloid-β may not be necessary.

Some stereological studies have suggested that there may be little or no neuronal loss during "normal" aging despite, as pointed out above, the presence of an increasing number of plaques [36]. Interestingly, even the hyperphysiologic levels of amyloid-β in mouse models of AD [27] only lead to senile plaque formation in middle-aged mice and, like their human counterparts, these mice show evidence of oxidative stress that precedes the amyloid-β deposits [18, 46, 48, 53]. Taken together, these findings indicate that amyloid-β is a *consequence* of the pathogenesis that serves an antioxidant function (Fig. 2).

The idea that amyloid-β is protective should not necessarily be surprising. Neuronal degeneration is associated with a number of responses including the induction of heat shock proteins [3, 51] that, like amyloid-β, show a relationship with cognitive decline. Yet only amyloid-β is considered pathogenic since amyloid-β is neurotoxic in vitro and is weakly associated with neuronal loss in vivo [5, 22]. On the other hand, as alluded to above, neurotoxicity in cultured cells may be an artifact of in vitro conditions [49], since neither isolated senile plaques nor immobilized amyloid-β elicit neurotoxity in vivo or in vitro [10, 17, 20] (Fig. 1). Thus, the capacity of amyloid-β to induce oxidative stress remains controversial [61] but may be akin to the known pro-oxidant effect of all antioxidants that is dependent on environmental conditions.

The few reports demonstrating neuronal loss in some transgenic AD models [8] argue that amyloid-β is a bioactive substance, but do not provide a compelling analogy to sporadic AD in humans. In addition, there is little evidence demonstrating behavioral deficits in mice transgenic for only AβPP mutations, while the most consistent deficits have been shown in mice transgenic for more than one mutation e.g., AβPP/presenilin 1 [25, 30], superimposed upon an aged environment.

The notion that extracellular amyloid deposits may not be harbingers of cell death recently found support from prion disease [13].

Tau

Tau accumulation in the form of neurofibrillary pathology may also represent oxidative imbalance [42]. According to recent studies, quantitative analysis of the extent of oxidative damage is *reduced* in those neurons with the most cytopathology [42]. Further studies suggest that most neuronal loss in AD occurs prior to NFT deposition [24, 33], a period associated with high levels of oxidative stress, while subsequent deposition of NFT decreases these levels [40].

The physiological modification of tau and neurofilament proteins by lipid peroxidation products and carbonyls is consistent with this view [50, 52]. Indeed, oxidative stress and attendant modification of tau byproducts of oxidative stress including HNE [59] and other cytotoxic carbonyls [9], enable such neurons to survive for decades [39]. Interestingly, although tau and neurofilaments, being cytoskeletal proteins, have a long half-life, the extent of carbonyl modification is comparable throughout the aging spectrum, as well as along the length of the axon [62]. A logical explanation for this finding is that the oxidative modification of cytoskeletal proteins is under tight regulation [35, 54].

A high content of lysine–serine–proline (KSP) domains on both tau and neurofilament protein suggests that they are uniquely adapted to oxidative attack. Exposure of these domains on the protein surface is effected by extensive phosphorylation of serine residues resulting

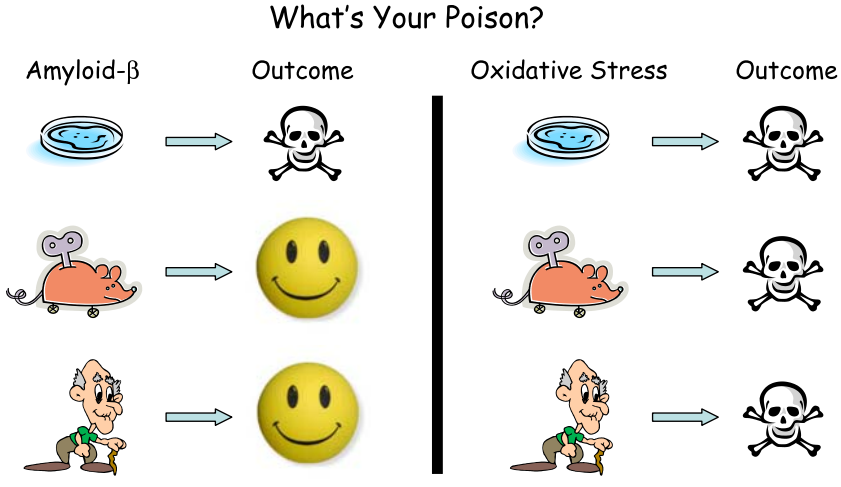

Fig. 1 Amyloid-β is toxic in vitro to cells in culture (Petri dish). However, amyloid-β does not cause toxicity in transgenic animal model nor in aged human brain. On the other hand, oxidative stress has deleterious outcomes in all in vitro and in vivo situations

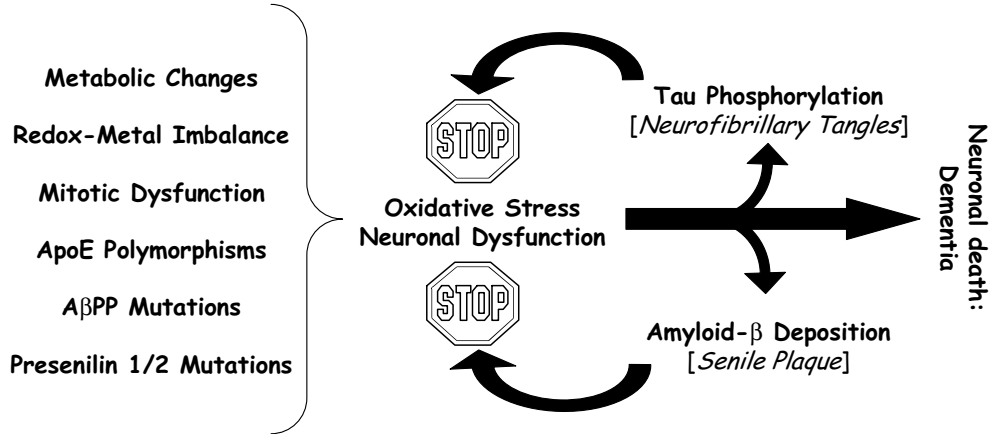

Fig. 2 Age-related increases in oxidative stress lead to increases in amyloid-β and tau phosphorylation, which both serve to attenuate oxidative stress. However, in AD, such age-related oxidative stress is magnified by a host of pathologic and etiologic aspects (metabolic changes, redox-metal imbalance, mitotic dysfunction, Apolipoprotein E polymorphisms, and AβPP and PS1/2 mutations), which overwhelm the compensatory increases in amyloid-β and tau phosphorylation and ultimately leads to neuronal death and dementia

in an oxidative "sponge" of surface-modifiable lysine residues [62]. Since phosphorylation plays this pivotal role in redox balance, it is not surprising that oxidative stress, through activation of MAP kinase pathways, leads to phosphorylation [68–70], nor that conditions associated with chronic oxidant stress, such as AD, are associated with extensive phosphorylation of cytoskeletal elements. Indeed, other neurological conditions where phosphorylated tau and neurofilament protein accumulations occur, also show evidence of oxidative adducts, e.g., progressive supranuclear palsy [45], corticobasal degeneration [11], and frontal temporal dementia [21]. Given this protective role, it is not surprising that embryonic neurons that survive treatment with oxidants have more phospho-tau relative to those that die [19]. Further, since heme oxygenase induction and tau expression are opposing [58, 59] indicating reduced oxidative damage in neurons with tau accumulation may be a part of the antioxidant function of phosphorylated tau (Fig. 2).

The concept that intracellular inclusions are manifestations of cell survival has recently found support in a Huntington's disease model [4]. In this neuronal model, cell death was mutant-huntingtin-dose- and polyglutamine-dependent; however, huntingtin inclusion formation correlated with cell *survival*. Thus, in this model, as in AD, inclusion formation represents adaptation, or a productive, beneficial response to the otherwise neurodegenerative process. Taken together with our studies, this represents a fundamental and necessary change in which pathological manifestations of neurodegenerative disease are interpreted.

Summary

The long-held notion that pathological lesions in neurodegenerative diseases provide direct insight into etiology may be a fundamental misconception. The observed decrease in oxidative damage with amyloid-β and tau accumulation suggests, rather, that senile plaques and neurofibrillary pathology are empirical manifestations of cellular adaptation (Fig. 2). Efforts aimed solely at eliminating amyloid-β or tau may therefore be directed against a biochemical process that is more physiological than pathological and therefore unlikely to produce the desired results. We further suggest that the classical notion of neurodegenerative disease pathology as signifying disease per se be re-organized into a modern framework that recognizes the difference between cause and effect [34]. Only through such an effort will the greatest potential for continued diagnostic and therapeutic advances be realized.

Acknowledgments Work in the author's laboratory has been or is supported by the National Institutes of Health, the Alzheimer's Association, and the John Douglas French Alzheimer's Foundation.

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