REGULAR PAPER

Hiroshi Choei · Nobuyuki Sasaki · Masayoshi Takeuchi
Taku Yoshida · Wataru Ukai · Sho-ichi Yamagishi
Seiji Kikuchi · Toshikazu Saito

Glyceraldehyde-derived advanced glycation end products in Alzheimer's disease

Abstract The Maillard reaction that leads to the formation of advanced glycation end products (AGE) is considered to play an important role in the pathogenesis of Alzheimer's disease (AD). Until now AGE derived from glucose (glucose-AGE) have been mainly investigated, so we established new AGE species derived from α-hydroxyaldehydes and dicarbonyl compounds. We have found that AGE derived from glyceraldehyde (glycer-AGE) and glycolaldehyde (glycol-AGE) showed strong neurotoxicity for primary cultured rat cortical neurons in vitro. In this study, we immunohistochemically examined the localization of glycer-AGE and glycol-AGE in the brains of AD patients and elderly controls. Most of the neurons in AD or control brains did not show any immunoreaction with glycol-AGE. In AD brains, glycer-AGE was mainly present in the cytosol of neuron in the hippocampus and para-hippocampal gyrus, but not in senile plaques and astrocytes. The pattern of immunopositivity was uniform and powdery, not dot-like. The distribution of glycer-AGE differed from that of glucose-AGE, which was detected at both intracellular and extracellular sites. This suggests that glycer-AGE has a pathological role different from glucose-AGE in AD. In the central nervous system, glyceraldehyde is generated via the glycolytic pathway from glyceraldehyde-3-phosphate by glyceraldehyde-3-phosphate dehydrogenase (GAPDH). We hypothesize that perikaryal glycer-AGE immunopositivity of neurons reflects an increase of cytoplasmic glycer-AGE along with the decline of GAPDH activity.

Keywords Advanced glycation end products · Alzheimer's disease · Glyceraldehyde · Glyceraldehyde-3-phosphate dehydrogenase · Glycolytic pathway

H. Choei · T. Yoshida · W. Ukai · T. Saito
Department of Neuropsychiatry,
Sapporo Medical University School of Medicine, Sapporo, Japan

N. Sasaki (✉)
Psychiatric Institute, Department of Psychiatry,
College of Medicine, University of Illinois at Chicago,
1601 W. Taylor St, Chicago, IL 60612, USA
e-mail: nsasaki@sapmed.ac.jp

M. Takeuchi
Department of Biochemistry, Faculty of Pharmaceutical Science,
Hokuriku University, Kanazawa, Japan

S. Yamagishi
Division of Endocrinology and Metabolism,
Department of Medicine, Kurume University School of Medicine,
Kurume, Japan

S. Kikuchi
Department of Neurology,
Hokkaido University Graduate School of Medicine, Sapporo, Japan

Introduction

Glucose and other reducing sugars react nonenzymatically with protein amino groups to initiate a post-translational modification process known as nonenzymatic glycation [2, 12]. This reaction proceeds from reversible Schiff bases to stable, covalently bonded Amadori rearrangement products. Once formed, the Amadori products undergo further chemical reactions to form irreversibly bound advanced glycation end products (AGE) [2, 12], which are considered to play an important role in the pathogenesis of the chronic complications of diabetes mellitus [2, 12].

Recent studies have suggested that there is a connection between Alzheimer's disease (AD) and AGE [11, 14] or the receptor for AGE [15]. AGE is a generic term for the advanced products derived from the reaction of sugars with proteins or amino acids [7, 12]. AGE have mainly been investigated in the field of diabetes mellitus, especially the AGE derived from glucose (glucose-AGE) [2]. Nevertheless, the process of AGE creation suggests that not only glucose but also various other sugars, including short chain sugars, can participate in the reaction that form AGE [5]. α-Hydroxyaldehydes and dicarbonyl compounds are produced from glucose in vivo. In previous studies [18, 20], we have shown that α-hydroxyaldehydes
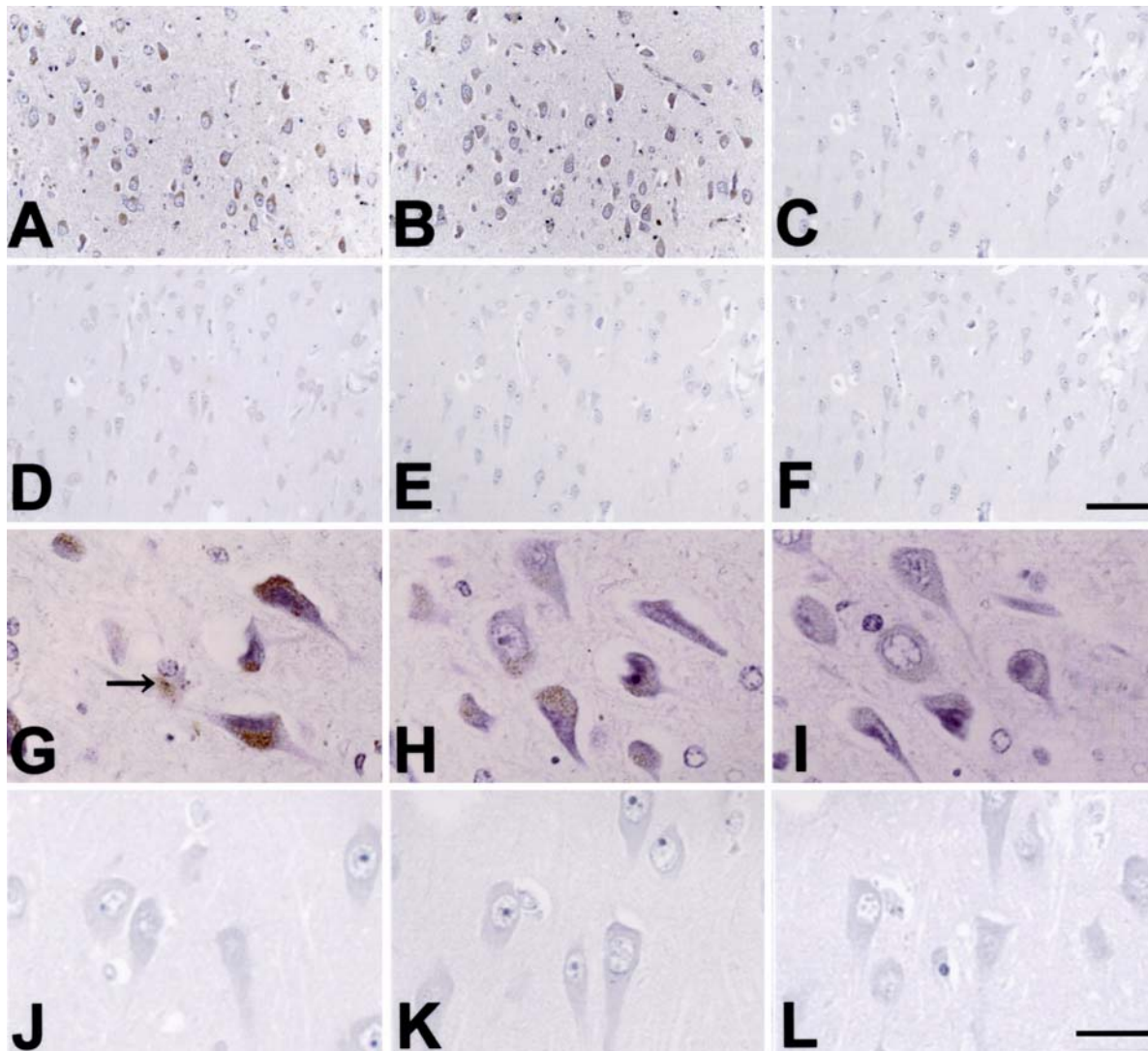
Fig. 1 Immunohistochemical staining of serial sections of the hippocampus from an AD brain (**A–C**, **G–I**), with glucose-AGE (**A**, **G**), glycer-AGE (**B**, **H**), and glycol-AGE (**C**, **I**) antibodies. Many glucose-AGE-immunopositive granules are seen in astrocytes (*arrow*, **G**) and the perikarya of the neurons (**A**, **G**). Powdery glycer-AGE immunopositivity is observed in the perikarya of the neurons (**B**, **H**). The glycol-AGE antibody shows negative or very weak staining (**C**, **I**). **D–F**, **J–L** Serial sections of the hippocampus from a control brain stained with glucose-AGE (**D**, **J**), glycer-AGE (**E**, **K**), and glycol-AGE (**F**, **L**) antibodies. The glucose-AGE, glycer-AGE and glycol-AGE antibodies show negative or very weak staining (*AD* Alzheimer's disease, *AGE* advanced glycation end products, *glucose-/glycer-/glycol-AG*E AGE derived from glucose/glyceraldehyde/glycolaldehyde, respectively). *Bars* **F** (also for **A–E**) 100 μm; **L** (also for **G–K**) 15 μm

(glyceraldehyde and glycolaldehyde) and dicarbonyl compounds (glyoxal, methylglyoxal, and 3-deoxyglucosone) make a contribution to the glycation of proteins. We have also established antibodies against AGE derived from these α-hydroxyaldehydes and dicarbonyl compounds [18].

Furthermore, we confirmed that AGE derived from glyceraldehyde (glycer-AGE) and glycolaldehyde (glycol-AGE) show strong neurotoxicity for primary cultured rat cortical neurons [19]. In vitro the neurotoxicity of these AGE species was stronger than that of glucose-AGE and *N*-(carboxymethyl)-lysine (CML), which are two AGE species that have been studied extensively. However, the existence of glycer-AGE and glycol-AGE in the human brain has not been investigated. In this study, we immunohistochemically examined the localization of glycer-AGE and glycol-AGE in the brains of AD patients and elderly controls.

Materials and methods

Subjects and specimens

Brain tissue specimens were obtained from eight patients with pathologically verified AD (age range: 45–68 years) and eight age-matched controls without dementia (age range: 49–73 years). None of the subjects was diabetic. Histological sections were prepared from the temporal cortex and the hippocampus.

Antibodies

Antibodies specific for AGE were prepared as described previously [18, 20]. Briefly, AGE-modified serum albumin was prepared by the incubation of rabbit serum albumin with D-glucose, D-glyceraldehyde, or glycolaldehyde. After immunization of rabbits, three types of AGE-specific antisera were obtained that were specific for glucose-AGE, glycer-AGE, and glycol-AGE. These antisera were subjected to affinity chromatography using CNBr-activated Sepharose 4B coupled to BSA modified by these three types of AGE. The eluted antibody was then further purified by CML-BSA affinity chromatography. An anti-amyloid β protein (Aβ) antibody, 4G8 purified IgG (Senetek PLC), was also used for immunostaining.

Immunohistochemistry

Serial paraffin sections were immunostained according to the standard streptavidin-biotin peroxidase technique using a Vectastain ABC elite kit (Vector Lab., CA). All sections were treated with 99% formic acid for 3 min, after which the sections used for AGE staining were also treated with 0.05% proteinase-K for 30 min. Endogenous peroxidase was inactivated by incubation with 0.3% H_2O_2 in methanol for 30 min and the sections were also incubated with 10% goat serum to eliminate nonspecific binding. This was followed by incubation overnight at 4°C with the primary antibodies diluted to 1:1,000 in 10 mM PBS (pH 7.4). Then the sections were sequentially incubated with the biotinylated secondary antibody for 1 h, with streptavidin-biotin-horseradish peroxidase for 1 h, and with 3,3′-diaminobenzidine/H_2O_2 for 1–3 min to develop the reaction products. Finally, the sections were counterstained with hematoxylin. The specificity of immunostaining was confirmed by applying PBS instead of the primary antibodies or by replacing the primary antibodies with non-immune serum.

Results

In AD brains, neurons and astrocytes showed strong staining by the glucose-AGE antibody (Fig. 1A, G). Many immunopositive granules were identified in the perikarya of the neurons, as reported previously [3], while the astrocytes contained characteristic immunopositive granules [8]. Staining with the glycer-AGE antibody was positive in most of the hippocampal and para-hippocampal gyrus neurons. This staining was localized to the cytosol and did not occur in the nucleus. Furthermore, the pattern of immunopositivity was uniform and powdery, with no dot-like immunopositive granules being observed (Fig. 1B, H). Most astrocytes were not stained, but a few were weakly positive. Most neurons showed no reaction with the glycol-AGE antibody (Fig. 1C, I), but a few showed very weak immunoreactivity.
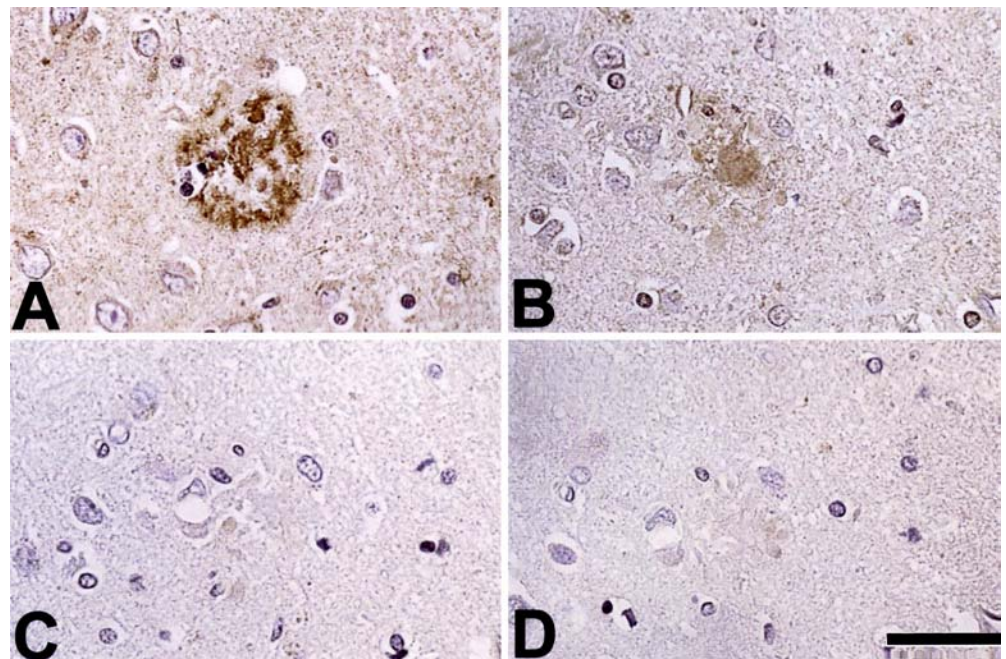
Figure 1D–F, J–L shows a control brain stained with the glucose-AGE, glycer-AGE, and glycol-AGE antibodies. The glucose-AGE antibody stained only a few neurons, which showed very weak immunoreactivity; it did not stain any structures in the astrocytes (Fig. 1D, J). Although neither neurons nor astrocytes were stained by the glycer-AGE (Fig. 1E, K) or glycol-AGE antibodies (Fig. 1F, L) in the controls shown, a small number of glycer-AGE-positive neurons were observed, but the staining was very weak compared to that in AD brains (data are not shown).

In the AD brains, many senile plaques were detected by Aβ immunostaining (Fig. 2A). The glucose-AGE antibody also reacted with the senile plaques, mainly with the amyloid core, but the glycer-AGE (Fig. 2B) and glycol-AGE (Fig. 3B) antibodies showed no immunoreactivity with the plaques.

Discussion

This immunohistochemical study showed that glycer-AGE was detectable in AD brains. It was mainly localized in the perikarya of neurons and the staining pattern was

Fig. 2 A–D Immunohistochemical staining of serial sections of the hippocampus from an AD brain, using antibodies for Aβ (**A**), glucose-AGE (**B**), glycer-AGE (**C**), and glycol-AGE (**D**). A senile plaque shows Aβ and glucose-AGE immunoreactivity (**A**, **B**), but is negative for glycer-AGE and glycol-AGE (**C**, **D**) (Aβ amyloid β protein). *Bar* 100 μm

Glucose

↓

Glucose-6-phosphate

↓

Fructose-6-phosphate

↓

Fructose-1,6-bisphosphate

↓

Glyceraldehyde-3-phosphate

GAPDH ———●

↓

1,3-Bisphosphoglycerate   **Glyceraldehyde**

↓   ⊢ Proteins
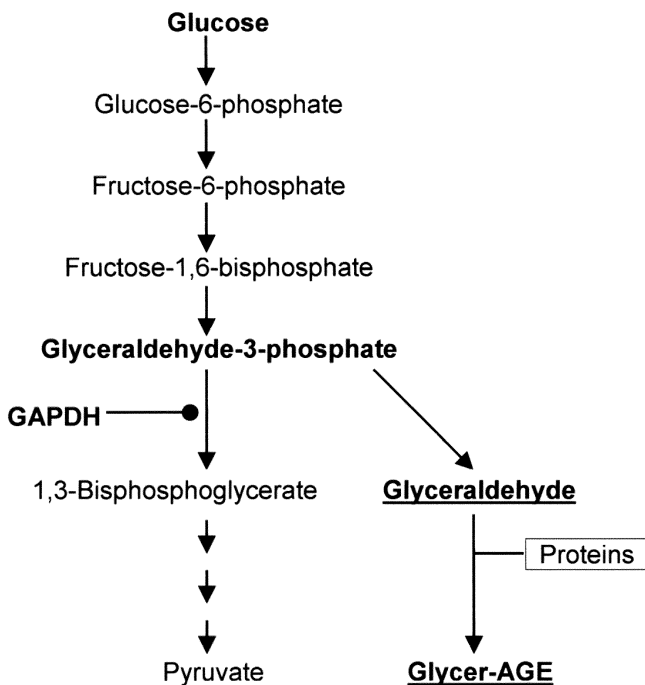
↓

↓

Pyruvate   **Glycer-AGE**

Fig. 3 The glycolytic pathway and glycer-AGE generation (*GAPDH* glyceraldehyde-3-phosphate dehydrogenase)

powdery, differing from the dot-like pattern of glucose-AGE staining. In contrast, this antibody stained only rare neurons in control brains, and this staining was very weak compared to that in AD brains.

In this study, we also investigated glycol-AGE because, like glycer-AGE, it shows strong toxicity for cultured cortical neurons. However, our results showed very little glycol-AGE positivity in either AD or control brains, which suggests that glycol-AGE may have little role in the central nervous system.

The present immunohistochemical study found no glycer-AGE in the senile plaques of AD brains. In contrast, many senile plaques contained glucose-AGE, suggesting that Aβ is glycated by glucose, rather than by glyceraldehydes. Glucose-AGE was present at both intracellular and extracellular sites, while glycer-AGE was only detected intracellularly. These results would suggest that the neurotoxicity of glycer-AGE is unrelated to glycation of Aβ.

Wong et al. [22] reported that AGE was localized in astrocytes and microglia with inducible nitric oxide (NO) synthase, and that this finding supports the activation of microglia and astrocytes by AGE-induced oxidative stress. In the present study, we identified glucose-AGE in astrocytes, but astrocytes containing glycer-AGE were rare. If oxidative stress mediated by glial cells is the main cause of AGE neurotoxicity, our finding is paradoxical. Since glycer-AGE has a far stronger neurotoxicity than glucose-AGE, detection of many astrocytes containing glycer-AGE would be expected. This discrepancy shows that the cause of neurotoxicity differs between glucose-AGE and glycer-AGE.

The relative browning activity, which reflects an early stage of the Maillard reaction, of glyceraldehyde and glycolaldehyde was about 2,000 times greater than that of glucose. In addition, glyceraldehyde and glycolaldehyde show a far stronger promotion of radical formation than glucose [20]. Usui et al. [21] reported that a glyceraldehyde-derived pyridinium compound depressed the intracellular glutathione level and induced reactive oxygen species production in the absence of glial cells. These findings suggest that glycer-AGE is associated with neurotoxicity induced by oxidative stress, but the main neurotoxic pathway is direct radical formation, which is not mediated by glial cells.

The energy supply for neurons in the brain is generated from glucose via the glycolytic pathway. As shown in Fig. 3, glyceraldehyde-3-phosphate is an intermediate in this pathway, undergong enzymatic reduction by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and eventually forming pyruvate. Some glyceraldehyde-3-phosphate is also transformed to glyceraldehyde, which reacts nonenzymatically with proteins to create glycer-AGE [16]. This glycolytic process occur in the cytoplasm where glycolytic enzymes exist.

In AD patients, positron emission tomography indicates that cerebral glucose metabolism is decreased, and this hypometabolism is seen from the early stage of AD [11]. Bigl et al. [1] reported that the activity of some glycolytic enzymes was abnormal in AD brains. They found that pyruvate kinase and lactate dehydrogenase showed a significant increase of activity in the frontal and temporal cortex, while the activity of glucose 6-phosphate dehydrogenase was significantly reduced in the hippocampus.

Ishitani et al. [6] reported that GAPDH may have an important role in neuronal apoptosis as a "killing protein" based on the overexpression of GAPDH in neurons during the apoptotic process, but this GAPDH is distinct from its role in glycolysis [10].

Mazzola et al. [9] reported that GAPDH activity showed a 27% decrease in AD fiblobrasts compared with control cells despite the overexpression of GAPDH, which they explained as occurring in compensation on for the decrease of GAPDH activity. Finch et al. [4] hypothesized that GAPDH is inhibited in AD, on grounds that the redox forms of NO inhibit GAPDH, and the combination of NO and superoxide anion generates peroxynitrite, a potent oxidizing agent implicated in inhibition of GAPDH by NO.

Along with the decline of GAPDH activity, the metabolism of glyceraldehyde-3-phosphate decreases and glyceraldehyde-3-phosphate accumulates intracellularly. Subsequently, the processing of glyceraldehyde-3-phosphate shifts to another route and the amount of glyceraldehyde is increased, which leads to an increase of glycer-AGE. The toxicity of glycer-AGE for cultured neurons is strong, and it promotes a marked increase of apoptosis. Accordingly, the perikaryal glycer-AGE immunopositivity of neurons in this study probably reflects an increase of glycer-AGE in the cytoplasm along with the decline of GAPDH activity.

References

1. Bigl M, Bruckner MK, Arendt T, Bigl V, Eschrich K (1999) Activities of key glycolytic enzymes in the brains of patients with Alzheimer's disease. J Neural Transm 106:499–511

2. Brownlee M, Vlassara H, Cerami A (1984) Nonenzymatic glycosylation and the pathogenesis of diabetic complications. Ann Intern Med 101:527–537

3. Crter J, Lippa CF (2001) Beta-amyloid, neuronal death and Alzheimer's disease. Curr Mol Med 6:733–737

4. Finch CE, Cohen DM (1997) Aging, metabolism, and Alzheimer disease: review and hypotheses. Exp Neurol 143:82–102

5. Glomb MA, Monnier VM (1995) Mechanism of protein modification by glyoxal and glycoaldehyde, reactive intermediates of the Maillard reaction. J Biol Chem 270:10017–10026

6. Ishitani R (1997) A roll for GAPDH in neuronal apoptosis. Seikagaku 69:1107–1111

7. Ledl F, Schleicher E (1990) New aspects of the Maillard reaction in foods and in the human body. Angew Chem Int Ed Engl 6:565–706

8. Li JJ, Surini M, Catsicas S, Kawashima E, Bouras C (1995) Age-dependent accumulation of advanced glycosylation end products in human neurons. Neurobiol Aging 16:69–76

9. Mazzola JL, Sirover MA (2001) Reduction of glyceraldehydes-3-phosphate dehydrogenase activity in Alzheimer's disease and in Huntington's disease fibroblasts. J Neurochem 76:442–449

10. Mazzola JL, Sirover MA (2003) Subcellular alteration of glyceraldehyde-3-phosphate dehydrogenase in Alzheimer's disease fibroblasts. J Neurosci Res 71:279–285

11. Meguro K, LeMestric C, Landeau B, Desgranges B, Eustache F, Baron JC (2001) Relations between hypometabolism in the posterior association neocortex and hippocampal atrophy in Alzheimer's disease: a PET/MRI correlative study. J Neurol Neurosurg Psychiatry 71:315–321

12. Monnier V, Cerami A (1981) Nonenzymatic browning in vivo: possible process for aging of long-lived proteins. Science 211:491–493

13. Namiki M (1988) Chemistry of Maillard reactions: recent studies on the browning reaction mechanism and the development of antioxidants and mutagens. Adv Food Res 32:115–184

14. Sasaki N, Fukatsu R, Tsuzuki K, Hayashi Y, Yoshida T, Fujii N, Koike T, Wakayama I, Yanagihara R, Garruto R, Amano N, Makita Z (1998) Advanced glycation end products in Alzheimer's disease and other neurodegenerative diseases. Am J Pathol 153:1149–1155

15. Sasaki N, Toki S, Choei H, Saito T, Nakano N, Hayashi Y, Takeuchi M, Makita Z (2001) Immunohistochemical distribution of the receptor for advanced glycation end products in neurons and astrocytes in Alzheimer's disease. Brain Res 888:256–262

16. Sirover MA (1997) Role of the glycolytic protein, glyceraldehyde-3-phosphate dehydrogenase, in normal cell function and in cell pathology. J Cell Biochem 66:133–140

17. Smith MA, Taneda S, Richey PL, Miyata S, Yan SD, Stern D, Sayre LM, Monnier VM, Perry G (1994) Advanced Maillard reaction end products are associated with Alzheimer's disease pathology. Proc Natl Acad Sci USA 91:5710–5714

18. Takeuchi M, Makita Z, Yanagisawa K, Kameda Y, Koike T (1999) Detection of noncarboxymethyllysine and carboxymethyllysine advanced glycation end products (AGE) in serum of diabetic patients. Mol Med 5:393–405

19. Takeuchi M, Bucala R, Suzuki T, Ohkubo T, Yamazaki M, Koike T, Kameda Y, Makita Z (2000) Neurotoxicity of advanced glycation end-products for cultured cortical neurons. J Neuropathol Exp Neurol 59:1094–1105

20. Takeuchi M, Makita Z, Bucara R, Suzuki T, Koike T, Kameda Y (2000) Immunological evidence that non-carboxymethyllysine advanced glycation end-products are produced from short-chain sugars and dicarbonyl compounds in vivo. Mol Med 6:114–115

21. Usui T, Shizuuchi S, Watanabe H, Hayase F (2004) Cytotoxicity and oxidative stress induced by the glyceraldehyde-related Maillard reaction products for HL-60 cells. Biosci Biotechnol Biochem 68:333–340

22. Wong A, Luth HJ, Deuther-Conrad W, Dukic-Stefanovic S, Gasic-Milenkovic J, Arendt T, Munch G (2001) Advanced glycation endproducts co-localize with inducible nitric oxide synthase in Alzheimer's disease. Brain Res 920:32–40