

Anti-AGEing defences against Alzheimer's disease

G. Münch*¹, B. Kuhla*, H.-J. Lüth*, T. Arendt* and S.R. Robinson†

*Neuroimmunological Cell Biology Unit, Interdisciplinary Center for Clinical Research (IZKF) Leipzig, Inselstrasse 22, 04103 Leipzig, Germany, and †School of Psychology, Psychiatry & Psychological Medicine, Monash University, Clayton, Victoria 3800, Australia

Abstract

Accumulation of insoluble protein deposits and their cross-linking by AGEs (advanced glycation end products) in the brain is a feature of aging and neurodegeneration, especially in AD (Alzheimer's disease). In AD, two types of fibrillar protein aggregates are present: extracellular deposits (plaques) consisting mainly of A β (β -amyloid peptide), and intracellular deposits (tangles) composed predominantly of microtubule-associated protein tau. Both plaques and tangles are modified by AGEs, which occurs particularly at lysine and arginine residues. Interaction of a synthetic amyloid plaque (fibrillar A β) with microglia leads to a strong pro-inflammatory response, indicating that priming of immune cells with β -amyloid potentiates their response to secondary stimuli such as AGE and cytokines such as interferon- γ . Formation of hyperphosphorylated and cross-linked microtubule-associated protein tau aggregates, especially tau dimers as the first step in tangle formation, can be induced *in vitro* by the combination of okadaic acid, a PP2A phosphatase inhibitor, and methylglyoxal. These results suggest that excess production of reactive carbonyl compound ('carbonyl stress') and subsequent AGE formation can contribute to cross-linking of protein fibrils and to pathological pro-inflammatory signalling, which all contribute to pathological changes and dementia progression in AD. However, the human brain has developed the glyoxalase system, a most effective defence system to scavenge small dicarbonyl compounds such as glyoxal and methylglyoxal. Very importantly, this system needs GSH as a rate-limiting cofactor. Since GSH is limited under conditions of oxidative stress and inflammation, supplementation with antioxidants such as lipoic acid, vitamin E or flavonoids could indirectly strengthen the anti-glycation defence system in AD. In addition, synthetic carbonyl scavengers and anti-inflammatory drugs could also be valuable drugs for the 'anti-glycation' treatment of AD.

Introduction

AD (Alzheimer's disease) is a multifactorial disease that involves progressive synaptic loss and neuronal death. This neurodegeneration has been linked to the presence of extracellular plaques of A β (amyloid β) peptide and to the occurrence of intracellular deposits (tangles) that are composed predominantly of microtubule-associated protein tau. It is noteworthy that the proteins in these deposits are frequently modified by AGEs (advanced glycation end products) (Figure 1). AGEs are found in many aging and pathological tissues, but are particularly prevalent in AD. Evidence suggests that AGE-cross-linked aggregates may contribute to the pathophysiology of AD by a variety of mechanisms.

A β and local inflammation

Plaques composed of fibrillar A β are generally surrounded and infiltrated by activated microglia and astroglia [1] (Figure 2). Glial cells are directly activated by the A β peptide,

and also by the presence of AGEs, with the former having an opsonizing effect on the latter. Thus treatment of microglia with synthetic fibrillar A β leads to a strong pro-inflammatory response that potentiates their response to secondary stimuli such as AGEs and cytokines (e.g. interferon- γ) [2]. Glia activated by A β or AGEs secrete a broad repertoire of pro-inflammatory agents, including NO, interleukin-1 β , interleukin-6, tumour necrosis factor- α and macrophage colony-stimulating factor, many of which are potentially cytotoxic. It has been suggested that the production of these agents by activated glia may be a critical factor that links neuronal degeneration to A β deposition [3].

Neurofibrillary tangle formation

Formation of hyperphosphorylated and cross-linked tau aggregates, especially tau dimers as the first step in tangle formation, can be induced *in vitro* by the combination of phosphatase inhibitors and reactive carbonyl compounds [4]. These results show that an excessive production of reactive carbonyl compounds, or a reduced rate of carbonyl detoxification ('carbonyl stress'), can lead to AGE-mediated cross-linking of protein fibrils. Carbonyl stress might therefore be a root cause of the pathological pro-inflammatory cascade that contributes to the pathological changes and dementia in AD.

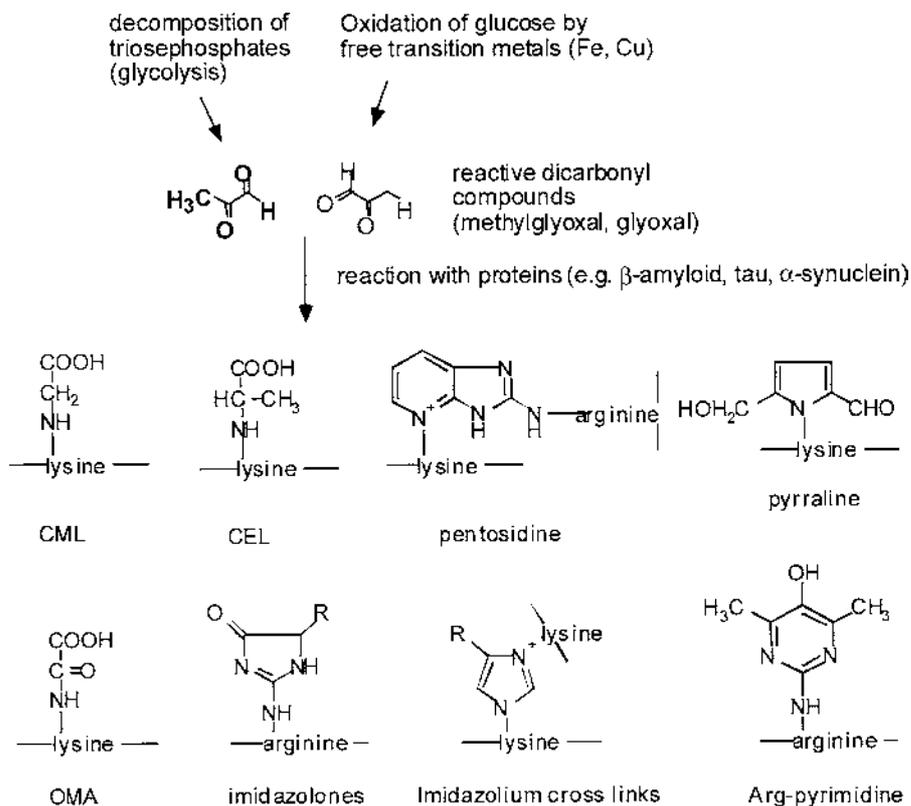
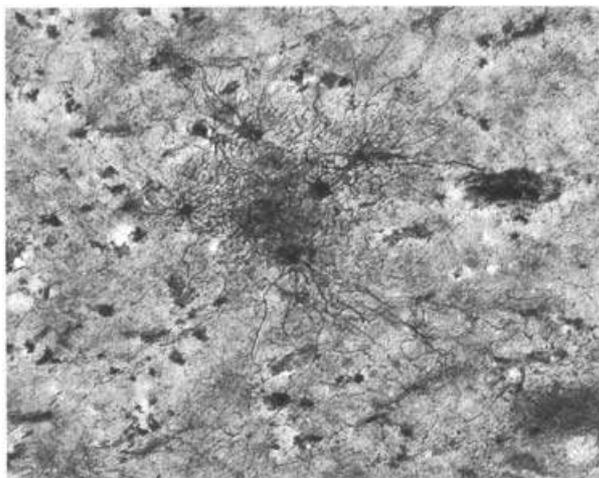
Key words: advanced glycation end product (AGE), Alzheimer's disease, antioxidant, glyoxalase, inflammation, oxidative stress.

Abbreviations used: AD, Alzheimer's disease; A β , amyloid β ; AGEs, advanced glycation end product.

¹To whom correspondence should be addressed, at the present address: School of Pharmacy and Molecular Sciences, James Cook University, Townsville, Qld 4811, Australia (e-mail Gerald.Munch@jcu.edu.au).

Figure 1 | AGEs – sources, reactions and chemical structures

CML, carboxymethyl-lysine; CEL, carboxyethyl-lysine; OMA, oxalic acid mono-*N*^ε-lysinyamide.

**Figure 2** | AGE immunoreactivity in activated glia surrounding Aβ plaques in AD-affected brain tissue

Anti-glycation defences

Organisms have developed a variety of anti-glycation defences that decrease the concentration of reactive carbonyl compounds. Such defences are present in the human brain. For example, the glyoxalase system is very effective at

scavenging small dicarbonyl compounds such as glyoxal and methylglyoxal which are then converted to D-lactate [5]. Very importantly, this system needs GSH as a rate-limiting cofactor. Since supplies of GSH are often limited under conditions of oxidative stress and inflammation, supplementation with antioxidants such as cysteine, lipoic acid, vitamin E or flavonoids could strengthen the anti-glycation defence system in AD.

Carnosine (β-alanyl-L-histidine), a dipeptide formed naturally in human tissues, is believed to inhibit the formation of cross-links between proteins that have been glycated. Carnosine is formed by a process involving the enzyme carnosine synthetase; this occurs mainly in muscles and brain. It is normally kept in equilibrium by the carnosinases, enzymes that inactivate carnosine in the tissues or in the blood. High concentrations of carnosine are present in long-lived cells (such as in neuronal tissues). The concentration of carnosine in muscles correlates with maximum lifespan, a fact that makes it a promising biomarker of longevity [6]. Specifically, carnosine reacts with and inactivates aldehydes and ketones, decreasing protein glycosylation and the rate of formation of AGEs. Because of its anti-glycation actions, carnosine supplementation may be useful in treating or preventing not only diabetic complications such as cataract, neuropathy and kidney failure, but also neurodegenerative diseases. Chemical inhibitors of the carnosinases are another therapeutic avenue worth exploring.

If the natural anti-glycation defences are indeed compromised in aging and in certain stress situations, synthetic carbonyl scavengers and anti-inflammatory drugs could become valuable drugs for the 'anti-glycation' treatment of AD. For example, the formation of new glycation-induced cross-links is slowed by aminoguanidine, which has been studied as an inhibitor of AGE-induced cross-links by Alteon Pharmaceuticals and is named Pimagedine. In animal experiment trials, Pimagedine slowed the progression of diabetic kidney disease and retinopathy [7]. Other agents that cap reactive amino groups, such as aspirin, may also inhibit the formation of AGE cross-links. While aminoguanidine and aspirin do not seem to break AGE cross-links that have already formed, other compounds are being studied that do. Many of the cross-link breakers are modified thiazolium salts that include an active site similar in structure to the catalytic ring of thiamine (vitamin B1). The most advanced compound in clinical development is 3-phenacyl-4,5-dimethylthiazolium chloride, named ALT-711 [8].

Summary

AGE cross-linking of proteins contributes to the pathogenic cascade of inflammation in AD. A variety of anti-glycation agents are currently being investigated as potential therapies

that might be able to slow the rate of neurodegeneration and the associated cognitive decline.

This work was supported by the Alzheimer Forschungs Initiative e.V. (AFI), the Bundesministerium für Bildung, und Forschung (BMB + F), Interdisziplinäres Zentrum für Klinische Forschung (IZKF) at the University of Leipzig (01KS9504; Project N1), and the EU (QLK6-CT-1999-02112).

References

- 1 Wong, A., Lüth, H.J., Deuther-Conrad, W., Dukic-Stefanovic, S., Gasic-Milenkovic, J., Arendt, T. and Münch, G. (2001) *Brain Res.* **920**, 32–40
- 2 Gasic-Milenkovic, J., Dukic-Stefanovic, S., Deuther-Conrad, W., Gärtner, U. and Münch, G. (2003) *Eur. J. Neurosci.* **17**, 813–821
- 3 Münch, G., Thome, J., Foley, P., Schinzel, R. and Riederer, P. (1997) *Brain Res. Rev.* **23**, 134–143
- 4 Perez, M., Hernandez, F., Gomez-Ramos, A., Smith, M., Perry, G. and Avila, J. (2002) *Eur. J. Biochem.* **269**, 1484–1489
- 5 Thornalley, P.J. (1998) *Chem.-Biol. Interact.* **111–112**, 137–151
- 6 Hipkiss, A.R. and Chana, H. (1998) *Biochem. Biophys. Res. Commun.* **248**, 28–32
- 7 Kern, T.S. and Engerman, R.L. (2001) *Diabetes* **507**, 636–642
- 8 Vasan, S., Foiles, P.G. and Founds, H.W. (2001) *Expert Opin. Invest. Drugs* **10**, 1977–1987

Received 11 June 2003