The role of the Maillard reaction in other pathologies: Alzheimer’s disease

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Abstract. Many approaches have and are being undertaken to treat Alzheimer’s disease but, as yet, no therapy is available with any established efficacy. Given the heterogeneity of the aetiological factors involved in Alzheimer’s disease and the difficulties encountered in the clinical diagnosis, the lack of pharmacological success is not surprising. Furthermore, the lack of an adequate animal model of Alzheimer’s disease has delayed the development of novel therapeutic strategies. At present, and with the exception of the rarer forms of familial Alzheimer’s disease, the need remains to treat the symptoms rather than the causes of the disease, primarily because the pathogenesis of Alzheimer’s disease is still unknown. The evidence for the role of glycation and advanced glycation end-products (AGEs) in the formation of neurofibrillary tangles and neuritic plaques, the characteristic histopathological lesions of Alzheimer’s disease, is briefly reviewed. While the role of glycation in the pathogenesis of Alzheimer’s disease is not yet unequivocally proven, it is the only single protein modification that would explain the formation of both the characteristic histopathological lesions first described by Alois Alzheimer in 1907. With our improved understanding of the molecular basis for the clinical symptoms of dementia, it is hoped that the aetiologically causes will afford more suitable targets for therapeutic intervention. In this respect it is interesting to note that the anti-inflammatory compounds indomethacin and acetylsalicylic acid, both inhibitors of the Maillard reaction, have been reported to have therapeutic potential and the nootropic agent tenilsetam inhibits protein cross-linking by AGEs.

Key words: advanced glycation end-products; Alzheimer’s disease; histopathological lesions; Maillard reaction

Aetiology and treatment of Alzheimer’s disease

Alzheimer’s disease is the most common single cause of dementia in late life, although its diagnosis can only be confirmed after autopsy. While 10\% of cases show a family history of the disease and are generally early-onset (mean onset age 40–60 years), the remainder are sporadic and late-onset (onset age after 60 years). Disease-causing mutations in three genes (the gene encoding for the amyloid precursor protein and two presenilin genes) have been discovered which account for nearly all of the rare familial, early-onset cases [1–3]. The sporadic, late-onset forms of the disease are associated with a number of genetic risk factors (apolipoprotein E, \(\alpha\)-antichymotrypsin, and very-low-density lipoprotein receptor polymorphisms and mitochondrial DNA mutations), but none of these is either sufficient on its own or necessary for the disease. This leaves open the possibility that one or more extrinsic environmental factors may affect the progression of Alzheimer’s disease. Such extrinsic factors might include head trauma, aluminium and antioxidants, although definitive evidence for their role is lacking. Alzheimer’s disease therefore is a multifactorial disorder [4], and the manner in which the different genetic and environmental factors interact to produce the neuropathology and dementia characteristic for the disease is not known.

Neuropathology of Alzheimer’s disease

Alzheimer’s disease is characterized by the pathological lesions first seen in conjunction in a patient by Alois Alzheimer: namely neuritic plaques and neurofibrillar tangles [5]. In addition to these hallmark features of the disease, several other lesions are found which have less disease specificity [6]. These include neuronal cell loss, activated microglial cells, astrogliosis and the presence of other intracellular bodies within neurons: granulovacuolar degeneration, incorporating abnormal isoforms of the microtubule-associated protein tau; Hirano bodies, which are composed predominantly of...
actin and actin-associated proteins; and the variable presence of Lewy bodies, composed of neurofilament proteins together with ubiquitin and its associated proteins. The pathology of Alzheimer’s disease is, however, best characterized by the presence of neurofibrillary tangles and neuritic plaques in the medial temporal cortex and other neocortical regions of the brain. Several studies have demonstrated that neurofibrillary lesions, which contain paired helical filaments (PHFs), are stronger indicators of clinical dementia than the extracellular deposits of amyloid-β-protein (Aβ) within plaques [7,8]. It is difficult to ascertain which of these three types of tau lesion contributes most in terms of neuronal dysfunction. Biochemical evidence indicates that a considerable PHF burden is found in neurites throughout the neocortex [9].

**Tau pathology**

Studies of Alzheimer’s disease have pointed to loss of the normal microtubule-associated protein tau [9], accumulation of pathological paired helical filaments [9] and loss of synapses in mid-frontal cortex [10] as strong discriminators for cognitive impairment. Tau is a microtubule-associated protein (MAP) that is involved in the assembly and stabilization of predominantly axonal microtubules, the integrity of which is required for the transport of essential material between the cell body and synapses of neurons. In the adult human brain, tau protein exists in six isoforms, ranging from 352 to 441 amino acids in length, that are derived by alternative splicing of mRNA encoded by a single gene located on chromosome 17q21 [11]. No mutations or polymorphisms in this gene have been identified. In Alzheimer’s disease tau protein forms the major integral constituent of the core structure of the PHF [12,13]. The proposed scenario is that the redistribution of tau protein into PHFs is associated with a failure of axonal transport in cortico-cortical association circuits arising from modifications of tau which inhibit its ability to maintain axonal tubulin in the polymerized state within pyramidal cells. The resulting failure of transport of synaptic constituents from projection soma to distant association neocortex would then lead to synaptic loss and cognitive impairment.

Tau proteins are susceptible to a number of post-translational modifications that may be important in the assembly of PHFs in Alzheimer’s disease. These include phosphorylation, ubiquitination, oxidation and proteolysis [11,14]. Despite a wealth of experimental data (predominantly *in vitro*) relating to these modifications, the temporal sequence with which these changes occur and the relative importance of specific changes remain uncertain, though much attention has been focused on phosphorylation [11,14]. Phosphorylation of tau protein suppresses its competence in the assembly of microtubules *in vitro* [11] and it appears that it is the extent of tau phosphorylation rather than the abnormal phosphorylation of disease-specific sites that is considered to be distinctive in Alzheimer’s disease [11,14]. The term ‘hyperphosphorylated tau’ is thus best used to describe some of the modified tau found in the brain [11]. However, recent studies indicate that only 5% of tau in PHFs is hyperphosphorylated, and this modification is thus not adequate to account for the substantial accumulation of PHFs found in Alzheimer’s disease [15]. Furthermore, fetal brain tau is hyperphosphorylated but does not form PHFs [11] and neurofibrillary tangles can develop in neurons in the absence of hyperphosphorylation [16]. Such studies suggest that phosphorylation of tau may be a secondary event in PHF formation.

Aggregation of tau *in vitro* can be induced by the action of transglutaminase [17]. Calcium-activated transglutaminase can catalyse the cross-linking of proteins into insoluble aggregates and does so by modifying the small number of glutamine residues in the C-terminal half of tau [17]. However, α-(γ-glutamyl)lysine isopeptide bonds have yet to be identified in PHFs or neurofibrillary tangles, and so the relevance of this modification *in vivo* remains to be proven. The histidine residues and the two cysteine residues in the tubulin-binding domain of tau are residues which are particularly prone to oxidative damage and such alterations could also lead to protein cross-linking [18]. Mandelkow and colleagues have proposed that phosphorylation at Ser262 leads to detachment of tau from microtubules and that oxidation of Cys322 leads to oligomerization of tau, and accelerates polymer aggregation and PHF assembly [18]. The relevance of tau oxidation to the pathology of Alzheimer’s disease, however, is still unknown, as is the mechanism of tau oxidation *in vivo* [19,20]. With respect to the latter, the proposal of protein glycation and the Maillard reaction as a physiological mechanism for the generation of oxidative moieties is particularly intriguing [14,19,21–23].

**Glyco-oxidation of tau protein in Alzheimer’s disease**

Protein glycation starts with the non-enzymatic, spontaneous condensation of a sugar aldehyde or ketone group with free amino groups, such as ε-NH₂ groups of lysine residues, to form a labile Schiff base, a reaction first described by Maillard in 1912. The resulting adducts then undergo irreversible rearrangements to form more stable Amadori or Heyns products and cross-linked advanced glycation end-products (AGEs) [22]. AGE-modified proteins are detergent-insoluble and protease-resistant, and have characteristic fluorescent spectra, brown pigmentation and modified amino acids, many features of which are shared by isolated PHFs from the brain tissue of Alzheimer’s disease patients [21]. Tau protein can be glycated *in vitro* and this inhibits its normal microtubule binding function (Figure 1) [24]. Interestingly, only the glycation of hyperphosphorylated tau, and not normal tau, results in the formation of fibrils that resemble PHF in
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vivo (Figure 2) [24]. The relevance of glycation as an additional pathological modification of tau in vivo is supported by the immunohistochemical co-localization of AGEs with both neurofibrillary tangles and PHFs [20,24]. Furthermore, PHF-tau isolated from the brains of Alzheimer's disease patients is glycated in the tubulin-binding region present in tau [25]. Finally, the introduction of glycated tau into cultivated cells can generate oxygen-free radicals capable of disturbing neuronal function [26].

The protease resistance and insolubility of PHFs isolated from neurofibrillary tangles suggests that the cross-linking of tau protein is important in their formation, and glycation/glyco-oxidation or trans-glutamination is therefore a potential pathological modification in Alzheimer's disease. It remains to be seen how important these changes are with respect to the accumulation of these and the other histopathological lesions observed in Alzheimer's disease, including the neuritic plaques, granulovacuolar degeneration, and Hirano and Lewy bodies which are also associated with other distinct types of dementia [6,41].

Aβ pathology

Besides the neurofibrillary tangles described above, the other characteristic feature of Alzheimer's disease is the presence of neuritic plaques in the medial temporal cortex and other neocortical regions of the brain [6]. The major constituent of the neuritic plaques is a protein, Aβ, which is a larger 39- to 43-residue-long peptide derived from a transmembrane amyloid precursor protein (APP). Aβ is deposited extracellularly throughout the cortex and often around blood vessels [6,27]. Mutations in the APP gene near to or within the beta-amyloid domain are linked with Alzheimer's disease in a few families throughout the world [1,28]. It has been proposed that APP mutations within and around the Aβ domain [28] lead to abnormal processing of APP and the accumulation of Aβ in the cortex, but this remains to be proven. Three pathways for processing APP have been identified so far, although the proteases implicated in these steps have not been identified [28]. There are two secretory pathways: one in which the Aβ domain is cleaved within the middle (α-secretase) and the second, a potentially amyloidogenic pathway, which leaves the Aβ peptide intact (β-secretase). APP can also be internalized and degraded via the endosomal/lysosomal pathway (γ-secretase) to yield C-terminal fragments of APP that also contain an intact Aβ domain. Recent studies have suggested that the ratio between Aβ peptides terminating at 40 and 42 or 43 residues is important, and that this may have an effect on the acceleration of
plaque formation [29]. The predominant forms of Aβ in diffuse and neuritic plaques tend to be those that terminate after 42 residues, in contrast to vascular deposits of Aβ, which tend to be 40 residues in length [30].

**Glyco-oxidation of Aβ in Alzheimer’s disease**

Besides abnormal processing, other modifications of APP may also play a role in amyloidosis and recent attention has focused on glycation as a potential pathological modification [31]. Vitek et al. have reported that AGE-modified synthetic Aβ can act as a "seed" for accelerating aggregation of soluble Aβ in vitro [32] and also found an increase in AGE adducts in the plaque core fractions from the cortex of Alzheimer’s disease patients compared with those from controls using an immunoassay specific for ‘late’ AGEs [32]. A role for glycation in plaque formation by Aβ in vivo is also supported by the immunohistochemical localization of Maillard products (pyralline and pentosidine) in both senile plaques and neurofibrillary tangles (NFTs) [33], although the lack of immunoreactivity observed for senile plaques with other anti-AGE antisera [34] emphasizes the need to define precisely the specificities of the antisera used. The latter point has been elaborated by the differences in immunoreactivity between antibodies recognizing free and protein-bound carboxymethyllysine at this meeting. Nonetheless, the insolubility of Aβ isolated from senile plaques suggests that covalent cross-linking is involved and modification by AGEs may combine with protein glyco-oxidation [31,23], oxidation [35] and the products of lipoperoxidation [33] to generate these lesions. Finally, with regard to the role of environmental factors in Alzheimer’s disease, it should be noted that aluminium-induced conformational changes to an Aβ fragment increase its susceptibility to glycation [36].

**Apolipoprotein E as a risk factor for Alzheimer’s disease**

As the late-onset, sporadic form of Alzheimer’s disease accounts for 90% of the cases, any potential pathological mechanism must be implicated in the disease process in the sporadic cases. One major genetic risk factor for the sporadic forms of Alzheimer’s disease is associated with the e4 allele of the apolipoprotein E (apoE) gene [37,38]. There is an e4 allele dose-dependent effect on the age of onset of Alzheimer’s disease: patients homozygous for the e4 allele have an age of onset that is ~15 years earlier than those lacking an e4 allele [39]. In addition, possession of an e4 allele is associated with increased susceptibility to cardiovascular disease [40], albeit to a lesser extent than for Alzheimer’s disease, with senile dementia of Lewy body type [41] and schizophrenia [42].

ApoE is a component of the very-low-density lipoproteins and is thought to play a key role in lipid trafficking [43]. There are three isoforms of apoE and these give rise to different apoE–lipoprotein complexes that can bind to at least three membrane-bound receptors [43,44]. In the CNS, apoE is believed to play a role in the mobilization and distribution of lipids in neuronal development [43]. In Alzheimer’s disease, APOE genotype can thus best be described as a genetic risk factor rather than a marker for the disease, and the role of apoE in pathogenesis remains an enigma. Poirier has suggested that apoE influences the disease by acting as a neuronal injury response protein in a mechanism implicating a role for inflammatory responses in the pathogenesis of Alzheimer’s disease [45].

**Apolipoprotein E and glycation**

A more direct role in the pathogenesis of Alzheimer’s disease is, however, suggested by two lines of evidence: apoE is associated with amyloid plaques and extracellular neurofibrillary tangles [46], and the apoE3 and E4 isoforms show opposite effects on neurite outgrowth in vitro [47]. With respect to the former, apoE has been shown to bind both Aβ [37] and tau [46] in vitro and is also found bound to Aβ in cerebrospinal fluid in vivo [48]. Although the pathological relevance of these interactions is unknown, the isoform specificity of these interactions [37,46] suggests that they are important in the aetiopathology of Alzheimer’s disease. The apoE3 and E4 isoforms differ by a single amino acid (Cys112 to Arg112), and the association of the apoE4 allele with Alzheimer’s disease has focused attention on functional differences between these isoforms [46]. In this respect it is interesting to note that though both isoforms of apoE4 bind to Aβ, only the e3 isoform binds to tau [46]. Furthermore, the binding...
of apoE3 to tau appears to be mediated by the microtubule-binding repeat domain as assessed by its ability to inhibit tau binding to microtubules (Figure 3), and the binding of apoE3 protects tau from glycation [Avila et al., in preparation]. These findings supports earlier results suggesting that glycation of hyperphosphorylated tau results in its assembly into PHFs [24].

With regard to the implication of glycation in the pathogenesis of Alzheimer's disease, the Cys112 to Arg112 change in the apoE4 allele introduces an intriguing change in the structure of the apoE molecule. In the apoE3 isoform, Cys112 is involved in a salt bridge interaction with Arg61, and its replacement results in the release of this Arg residue and its exposure to the external surface in the apoE4 isoform [44]. As the most commonly seen AGE cross-link in glycated proteins is pentosidine, which is formed by the cross-linking of lysine to arginine residues [22], the external availability of Arg61 for cross-linking by AGEs may be important in the generation of the apoE-containing histopathological lesions in Alzheimer's disease. Finally, with respect to the differential effect of the apoE isoforms on neuronal cells in vitro, it is interesting to note that apoE3 induces neurite outgrowth whereas the E4 isoform inhibits outgrowth and spreading [47]. Neurite outgrowth is also stimulated by the binding of the neuronal growth factor amphoterin to its neural cell surface receptor, which has recently been shown to be identical to the cell surface protein RAGE that had previously been described as the receptor for AGEs [49]. This raises the intriguing possibility that the binding of AGEs to neuronal RAGE may inhibit the binding of its physiological ligand amphoterin and thus inhibit neurite outgrowth. The relevance of these potentially pathological mechanisms to the development of Alzheimer's disease, however, remains to be elucidated, but they could account for the genetic risk associated with the apoE4 allele in the sporadic form of the disease.

Concluding remarks

The implication of glycation in the pathogenesis of Alzheimer's disease has opened up a number of new avenues in Alzheimer's research. Besides the relevance to understanding the neuropathology of the disease, the involvement of glycation focuses attention on disorders of metabolism, particularly of glucose, as the primary defects in Alzheimer's disease, and this is supported by the defects in glucose metabolism observed early in the disease using positron emission tomography [50]. This not only sheds new light on the pathogenesis of Alzheimer's disease but also offers the prospect of using such non-invasive techniques for both early diagnosis and objective evaluation of therapeutic regimes.

With respect to the involvement of glycation in other human diseases, glycation may be a general mechanism of amyloidosis [31] and may thus be involved not only in a number of neuropathological disorders but also in other diseases in which amyloid occurs, such as type II diabetes, where the formation of pancreatic amyloid is a feature of the pathology. Furthermore, with respect to diabetes, the prospect of inhibiting the processing of proinsulin to insulin by modification of the amino acid residues in the Lys–Arg and Lys–Lys endopeptidase cleavage sites may explain the high concentrations of plasma proinsulin observed early in the disease.

Finally, the most intriguing biological implication of glycation may be the consequence of its emerging role as a ubiquitous fundamental reaction between reducing sugars and protein. As cell surface and secreted proteins would be constantly exposed to plasma glucose, it is likely that the enzymatic glycosylation of these proteins is a defence mechanism to protect them against glycation. This hypothesis is supported by the recent report of the first structure of a cell surface protein plus N-glycan, which shows that the glycan moiety interacts with a cluster of five lysines on the surface of the polypeptide [51], the relevance of which is clear to any AGEists.

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