

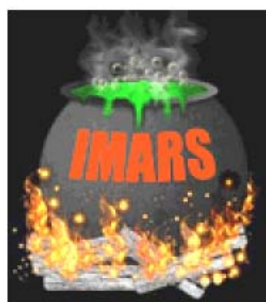
IMARS Highlights

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Editorial comment

In this issue of IMARS Highlights I have included a feature length article “Role of AGE in Alzheimer’s disease and vascular dementia” by a distinguished neuropathologist Professor Margaret Esiri from Oxford University. We are working towards publishing high standard articles and advising our editors to contribute more novel findings or detailed good reviews. IMARS is committed to provide a journal publishing reliable and good quality work. In this issue the selected short articles by our usual contributing editors – John Bayens, Monika Pischetsrieder, Alan Stit and Varoujan A. Yaylayan have been included. I would like to encourage readers to submit their new findings to the journal. In future there will be invited reviews by distinguished clinical and basic scientists, such as Margaret Esiri working on the periphery of glycation research giving expert views of both glycation processes and their likely consequences. I hope reading fine reviews by these top scientists will stimulate IMARS members to support their journal and help it become a reliable source of information on glycation and Maillard reaction research. I will keep encouraging IMARS members and readers to send their articles to the IMARS editorial board. As always feedback from readers on these and other features in this issue are very welcome.

This issue also brings you the first announcement of the young scientists essay competition for the 9th IMARS meeting in Australia in September 2009.

Naila Rabbani

n.rabbani@warwick.ac.uk

Role of AGE in Alzheimer's disease and vascular dementia

Margaret M Esiri, Departments of Neuropathology, Oxford University

Introduction

Ageing of the brain is a subject of intense topical interest in view of the demographic changes that have taken place since the early twentieth Century, and which have resulted in many more people living into old age. Pathological changes that occur in the brain with ageing are principally centred on the neurons and in the blood vessel walls, although there are additional changes also found in glial cells (1). The neuronal and vascular changes give rise to those diseases most closely associated with ageing: Alzheimer's disease (AD), Parkinson's disease and cerebrovascular disease (CVD). The latter manifests as strokes or as vascular cognitive impairment or dementia, largely depending on the size of vessel affected (large vessel disease generally gives rise to strokes and small vessel disease to cognitive impairment). Although there are rare cases of all these conditions that can occur at a young age due to gene mutations the vast majority of cases of diseases of this sort occurs sporadically and we are still ignorant of their respective pathogenesises. In this brief review I take a look at the evidence for a role for advanced glycation end products (AGE) in AD and vascular dementia.

A role for AGE in Alzheimer's disease?

Sporadic AD is by far the commonest neurodegenerative disease and is found at increasing prevalence and incidence as people enter their 70s and 80s such that people in their mid 80s have been estimated to have up to a 50% chance of having the condition. The pathology is complex with insoluble deposits of different proteins being found at extra- and intra-cellular sites (2). The extracellular deposits are composed principally of *beta amyloid*, a peptide derived by proteolytic cleavage of a larger *amyloid precursor protein (APP)* which is predicted from its structure to be a receptor but whose function is unclear. The intracellular protein consists of the microtubular-associated protein *tau*, a protein whose normal function is to provide a scaffold between microtubules and neurofilaments and in so doing help maintain the neuronal cytoskeleton. In AD tau forms distinctive *neurofibrillary tangles* in neuronal cell bodies, neuropil threads in the neuropil and neuritic processes that are found caught up with the beta amyloid protein in the extracellular so-called senile, or argyrophilic *plaques*. According to the *amyloid cascade hypothesis* of AD pathogenesis (3), based on the finding of an AD causative mutation in the *APP* gene (4), and also on the consecutive appearance of amyloid followed by tangles in the brains of Down Syndrome sufferers (5), amyloid formation is thought to initiate the pathogenesis of AD and lead subsequently to tangle formation. This sequence of events is supported by studies of transgenic mice with inserted APP and tau mutations (6). However, this process applies most convincingly to genetically determined AD and may be less wholly applicable to sporadic AD. Other strands of evidence link AD with altered expression of cell cycle control proteins and free radical damage (7,8).

Apart from increasing age, the risk factors for sporadic AD include a specific isoform (epsilon 4) of the serum protein apolipoprotein E (9) and various factors that are also risk factors for cardiovascular disease including hypertension in middle age, elevated levels of plasma homocysteine and cholesterol, and diabetes mellitus (10,11).

Protein glycation is the non-enzymatic, time-dependent, reaction of reducing carbohydrates, particularly glucose and its autoxidation products glyoxal, methylglyoxal and 3-deoxyglucose, with protein amino groups on amino acid side chains. With increasing age this can occur at normal glucose concentrations but it is enhanced if blood glucose levels are elevated, as in diabetes mellitus. In long-lived proteins the early products of glycation can further evolve irreversibly into AGE. When bound to their receptor, RAGE, AGE can evince an inflammatory reaction and free radical damage to neighbouring structures. Glycation of proteins leads eventually to increased intermolecular cross-linking, impairment of protein function and alteration of protein degradation. Evidence linking brain ageing and AD with protein glycation emerged in the 1990s with the demonstration of the receptor for advanced glycation end products (RAGE) in AD lesions (12) and increased expression of RAGE and AGE in brains from elderly subjects and in AD (13, 14). Neurons, astrocytes and microglial cells were identified as cells within, or around which, AGE accumulated. Beta amyloid has been identified as a ligand for RAGE and glycation of beta amyloid *in vitro* enhances its predisposition to aggregate. Over-expression of RAGE by neurons enhanced behavioural deficits and neuropathological changes in mutant APP transgenic mice. Conversely, RAGE null/mutant APP transgenic mice showed less AD pathology than mutant APP mice (15). This evidence suggests that RAGE (whose ligands are not restricted to AGE) can enhance any neurotoxicity possessed by beta amyloid.

AGE, being a heterogeneous collection of different compounds, could well have different effects on the brain, not all of them necessarily pathological. Thus, there has been a subdivision suggested into toxic and non-toxic AGE (16). Using anti-AGE antibodies Sato et al (16) recognised 6 classes of AGE in serum from type 2 diabetics undergoing haemodialysis. Of these, AGE-2, derived from glyceraldehyde, was identified as a neurotoxic component of diabetic serum when applied to neuronal cells *in vitro*. This AGE-2 neurotoxic effect was blocked by AGE-2 antibody. In human brains from people with AD the AGE-2 antibody identified fine staining in neuronal perikarya in the hippocampus but no staining in plaques whereas antibody to glucose-derived AGE-1 produced staining of plaques and neuronal staining that was coarser than that for AGE-2. Clearly there needs to be further analysis of such *in situ* staining for different AGE in AD and ageing as well as further work to investigate the source of AGE in the brain in AD (locally produced by neural/glial/endothelial cells containing them or derived from blood?) and the more downstream effects of increased AGE levels in brain (upregulation of soluble or membrane-bound RAGE, altered levels of AGE in body fluids or increased risk of altered proteins provoking autoimmunity?).

A role for AGE in vascular cognitive impairment and dementia?

Vascular cognitive impairment (VCI) is generally considered to be the second most common cause of cognitive impairment or dementia after AD although prevalence and incidence figures for VCI are much lower than for AD and are anyway less reliable because the criteria for diagnosis are less well defined and still evolving. Furthermore, many autopsy studies have emphasised the high frequency with which both AD and vascular disease co-exist in the brains of elderly demented subjects at autopsy. In the community-based Cognitive Function and Ageing study in England and Wales vascular disease was the only condition apart from AD which correlated with a history of dementia during life (17). The type of cerebrovascular disease that most frequently underlies VCI and vascular dementia (VD) is damage to the small, subcortical arteries and arterioles that supply blood and nutrients to the cerebral white matter, basal ganglia and thalamus (18). These vessels develop greatly thickened walls containing collagen, which replaces the smooth muscle of the tunica media. They also have a

reduced lumen size and are surrounded by enlarged perivascular spaces. The ease with which non-invasive brain imaging can now be undertaken has made it clear that this is a very common form of vascular pathology in the elderly and not always associated with cognitive impairment or dementia – indeed, it may be asymptomatic. Traditionally it has been linked to longstanding hypertension but it is certainly not limited to hypertensive subjects. In the territories of supply of the affected vessels there is axon loss as well as damage to the myelin sheaths of axons. It is this damage that is thought to give rise to cognitive impairment, by interrupting connections between nerve cells participating in neural networks subserving cognitive functions. The initial symptoms of VCI and VD are less stereotyped than in AD which usually commences with memory impairment. In VCI and VD the presentation is more varied with executive slowing of responses, motor problems and general forgetfulness which probably reflect more widely distributed pathology at the start of the pathological process than is the case in AD in which tangle formation (which bears a close relationship to cognitive impairment) commences in the medial temporal lobe and hippocampus, regions intimately related to memory function.

It was a pathological similarity between the pathological changes in small subcortical blood vessels in VCI and VD and those seen in end-organs damaged by diabetes mellitus that led to my interest in AGE in VD. Furthermore, a recent systematic review of 25 prospective observational studies identified a 2.2-3.4 times greater risk of VD in people with diabetes than in non-diabetics (19). This suggests that similar processes to those that cause end-organ failure in retina, kidney and peripheral nerve in diabetics may also operate to cause VCI and VD in the elderly. No long term prospective studies of risk factors for impaired cognition in diabetes are available but it has been noted that people with and without diabetes demonstrate an association between retinal microvascular abnormalities and cognitive function (20).

We set out to investigate expression of the AGE CML (N-epsilon-carboxymethyl lysine) in blood vessel walls in autopsy material from cases included in the Oxford Project to Investigate Memory and Ageing (OPTIMA). This is a longitudinal study of demented and undemented elderly subjects who undergo yearly clinical and psychological assessment while alive, with the great majority of subjects also generously consenting to have their brains examined neuropathologically after death. From the 300+ cases whose brains have been examined in this study we selected only those cases that had strong evidence of the type of small vessel disease described above and no significant other pathology that would explain dementia, if present. Our aim was to see if expression of AGE in vessel walls might be more abundant in those cases (n=10) that had dementia or cognitive impairment than in those that were cognitively intact (n=15).

Once we saw the tissue sections of frontal cortex and basal ganglia that had been immunostained for CML it was clear that there was a greater intensity of staining in some of the neurons than in the blood vessel walls so we analysed the proportion of neurons that expressed CML as well as giving vessel wall staining a semi-quantitative score for CML staining. We then analysed these results with respect to dementia status, whether or not the subject had suffered from hypertension during life, age at death and whether there had been a history of diabetes mellitus. We found that cortical neuronal staining for CML was significantly higher in the group of cases that had been demented than in the group of cases that had had normal cognitive status. It was also higher in those that had been hypertensive than in those that had been normotensive. The semi-quantitative scores for vessel staining for CML significantly related to cognitive impairment and also to a history of diabetes. Neuronal

staining for CML in the basal ganglia related to a history of hypertension and to lower age at death. These results have recently been published (21).

This is a pilot study that needs independent confirmation but the results are of interest in that they suggest that CML expression in the brain is related to clinically significant functional brain failure in old age. We chose to examine expression of CML because it is one of the most abundant AGE in tissues. However, as a glucose-derived AGE CML did not fall into the toxic category of AGE identified by Sato et al (16) and there is some evidence that CML reflects lipid peroxidation as well as glycooxidation. Clearly it would now be of interest to study a wider range of AGE in vascular dementia.

It is of interest that in our study (21) expression of CML related to two of the conditions that are associated with brain failure in the elderly – hypertension and diabetes. AGE formation is well established to be increased in diabetes but it may be noted that AGE formation has also been found to be greater in experimental hypertension in rats than in normotensive rats (22-25).

We are unable to tell from this study whether the excess CML expression in neurons was a cause or a consequence of brain injury but given the strong evidence that increased AGE production and its binding to RAGE in vessel walls in experimental diabetes causes end-organ damage in that condition it is at least a strong possibility that the same may be true of VCI and VD in the elderly. However, much further work will be needed to demonstrate that this is so.

Conclusions

In the two most common and important forms of brain damage in the elderly – AD and CVD – evidence is increasing that AGE play a significant role. AGE effects are likely to be one of a number of different factors promoting brain failure in old age but one that deserves increased attention if only because AGE effects may be amenable to novel and as yet unexplored therapies or preventive strategies to promote healthy brain ageing.

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Address for correspondence: Prof MM Esiri,
 Neuropathology Dept., Level 1, West Wing,
 John Radcliffe Hospital, Oxford OX3 9DU

e-mail: Margaret.esiri@clneuro.ox.ac.uk
 Tel 01865 234403

Oxidation, carboxyethylpyrrole (CEP) and inflammation – a chain leading to serious retinal disease

Anna M Pawlak, Alan W Stit, Queen's University Belfast

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in the Western world¹. The pathology of this complex disorder is closely linked to dysfunction of a specialised layer of retinal cells called the retinal pigment epithelium (RPE) and the underlying extracellular matrix called Bruch's membrane (BM).

The retina is concomitantly exposed to high oxygen partial pressure and blue radiation and the evoked oxidative pathways can modify polyunsaturated fatty acids (PUFA) in the photoreceptors. Indeed, oxidative stress along with inflammatory processes have been long considered as the important mechanisms contributing to AMD onset and progression². Advanced glycation and lipoxidation end-products (AGEs/ALEs) have been shown to accumulate with age in the retina and these products of Maillard chemistry are considered as possible modulators/mediators in AMD pathophysiology³.

The lipoxidation adduct carboxyethylpyrrole (CEP) is derived from the oxidation of docosahexaenoic acid (DHA) (Fig.1) and this PUFA constitutes ~50% of the photoreceptor

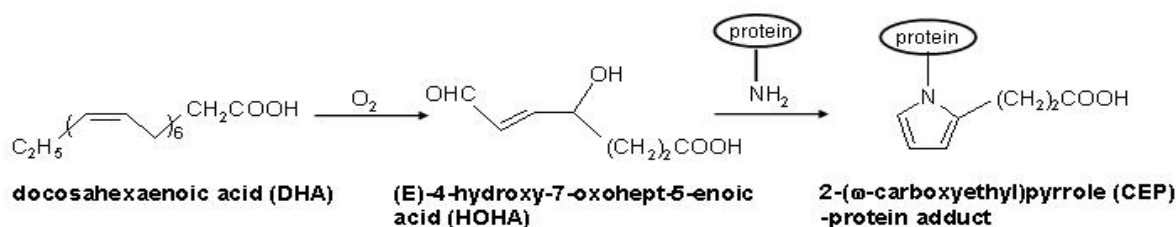


Fig.1 Formation of 2-(w-carboxyethyl)pyrrole (CEP) protein adduct. CEP is unique adduct arising only by oxidation of docosahexaenoic acid (DHA) to 4-hydroxy-7-oxohept-5-enoic acid (HOHA), which in turn reacts with protein.

phospholipids. CEP adducts are abundant in post-mortem AMD retinas, particularly in pathogenic sub-RPE deposits called “drusen”. CEP auto-antibody titres in plasma are also comparatively higher in AMD patients than in age-matched controls⁴. The link between ALEs and AMD pathogenesis has been recently strengthened by Holyfield *et al.* who immunized mice with CEP-modified mouse albumin (CEP-MSA) or native MSA in a short- and long-term experimental protocol⁵. The mice demonstrated significant levels of CEP-antibodies and, importantly, retinal analysis revealed lesions akin to features of AMD pathology, including thickening of BM, RPE and photoreceptor apoptosis, activation of complement and inflammatory cell infiltration. The degree of pathology revealed close relationship to the mean antibody titer, which was ~8 times higher in case of severe RPE pathology compared to the normal control⁵.

This study shows the first time that immunization with ALE adducts arising from DHA oxidation is sufficient to induce key AMD-like lesions in the mouse retina. It also provides a strong indicator that ALE adducts may link oxidative stress and inflammation involved in a cytotoxic chain reaction leading to significant pathology. Beyond this, Holyfield *et al.* highlight an important role for ALEs in a major age-related disease and may indicate future possibilities for pharmacological or even dietary intervention.

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Maillard-like reactions of 2-deoxy-ribose-5-phosphate

Varoujan A. Yaylayan, Department of Food Science & Agricultural Chemistry, Quebec, Canada

In a recent study (1) by Munanairi et al, on the kinetics and chemistry of ribose and deoxy-ribose phosphate reactions with different amino acids, the authors have confirmed the previously known catalytic role of phosphate groups in enhancing the initial rate of the Maillard reaction (orders of magnitude) and the increased formation of carboxymethyllysine (CML) from ribose under oxidative conditions. Interestingly, they also identified two pyrrole derivatives (**1** and **2** in Figure 1) specific to 2-deoxy-ribose-5-phosphate model system heated at 37°C and at pH of 7.5. The 2-deoxy-sugars are known to be unreactive in the classical Maillard reaction, however, it was proposed (2) that they can undergo dehydration and form 4-hydroxy-alkenal moieties capable of undergoing vinylogous Amadori rearrangement (vARP) with amino acids. The formation of the two pyrrole derivatives could be explained by the formation of such vinylogous Amadori products A and A'. Both can undergo a second reaction with amino acids at different positions. In A' the carbonyl group being conjugated, the amino acid preferably attacks the C-5 that contains a good leaving group phosphate and in A the carbonyl group being isolated, amino acid can easily form a Schiff base as shown in Figure 1. The intermediates formed from A and A' after the second amino acid reaction, can be eventually converted into pyrroles **1** and **2** through known transformations including oxidation. The significance of such intermediates lies in their ability to cross-link proteins if the two amino groups are located on different proteins.

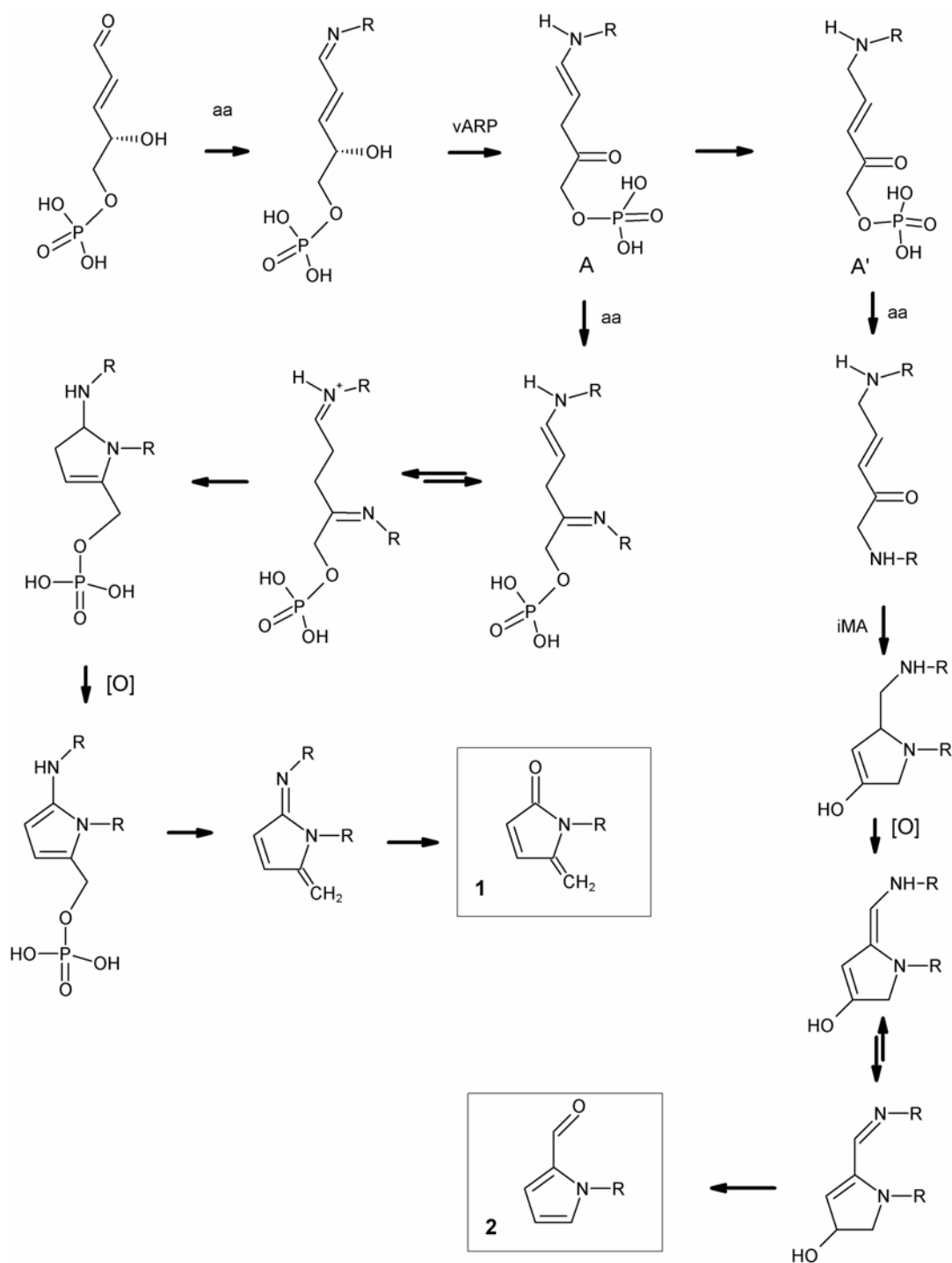


Figure 1. Proposed mechanism of formation of two pyrrole derivatives 1 and 2 from the reaction of amino acids with 2-deoxy-ribose-5-phosphate at 37°C and pH of 7.4

R= -CHR'COOH (amino acid residue); aa = amino acid, vARP = vinyllogous Amadori rearrangement, iMA = intramolecular Michael addition. [O] = oxidation

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Stem cell therapy for diabetic vascular complications ? not until we consider the consequences of advanced glycation

Alan Stitt, Queen's University Belfast, Northern Ireland

An interesting area in stem cell research is exploration of the therapeutic potential of bone marrow-derived endothelial progenitor cells (EPCs) (1). These stem cells can be mobilised as a natural response to tissue injury and ischaemia and then repair damaged vascular endothelial monolayers and defunct vessels. Once mobilised into the bloodstream, EPCs make an important contribution to organ regeneration by homing to sites of damage and promoting repair and, in some cases, re-perfusion of ischemic tissues by participation in neovascularisation. EPCs can be isolated from peripheral blood, umbilical cord blood or bone marrow, although an accurate definition and characterisation of the various EPC sub-populations is still required. Recently there has been a widely accepted consensus that *bona fide* circulating EPCs express surface markers shared by both endothelial (CD31, VEGFR2) and stem/progenitor (CD34, CD133) cell phenotypes.

A range of exciting clinical studies are now indicating that EPCs can be harnessed therapeutically to improve blood supply to inadequately perfused tissues (1). However in patients with diabetes it has been consistently demonstrated that CD34⁺ EPCs are dysfunctional, displaying impaired homing to damaged vessels and depressed recruitment into sites hypoxia and ischaemic injury (2). Of relevance to IMARS members is the discovery that EPCs show significantly impaired attachment, differentiation and incorporation into resident endothelial cell monolayers when the underlying vascular BM is AGE-modified (3).

A recent article by Ceradini *et al* has taken the concept of diabetes-linked EPC dysfunction a significant step further (4). The transcription factor HIF-1 α is a key regulator of hypoxia-induced growth factor / cytokine expression and the authors hypothesised that increases in the reactive dicarbonyl methylglyoxal (MG) within diabetic EPCs can AGE-modify HIF-1 α with downstream consequences for cell function. Ceradini *et al* demonstrated that in high glucose-exposed CD34⁺ EPCs, there was enhanced glycolytic metabolism, superoxide generation and MG-derived modifications of HIF-1 α . Using point mutation analysis in the bHLH domain of HIF-1 α , they defined the arginine residue that was susceptible to MG-modification (Arg-17) and then showed that this significantly inhibited DNA-protein binding efficiency. This impaired transcriptional activity in EPCs subsequently reduced CXCR4 expression (the receptor for SDF-1) and eNOS; both of which are important for progenitor immobilisation and recruitment. The key role of MG in this process was further underlined by protection against high glucose-related dysfunction by over-expression of glyoxalase-1 in the EPCs. The authors linked these discoveries to the attenuated vasculogenesis in response to soft tissue ischaemia in diabetic mice (4).

These findings demonstrate an important pathway that partially explains EPC dysfunction in diabetes. Ceradini *et al* show that AGE adduct formation has pathophysiological consequences, not just in organs traditionally viewed as “targets” of the diabetic milieu but also in the reparative, infiltrating cells that are derived in the marrow (4). More research is needed as we move towards cell therapy and the possibility that the inherent reparative capacity of EPCs could be harnessed for diabetic complications. If we can correct a diabetes-mediated defect in EPCs isolated from patients using an *ex-vivo* strategy, there is the possibility of re-introducing “fixed” autologous cells back into patients where they would regenerate damaged vasculature. Maybe we are not so far away!

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The AGE breaker myth

John Baynes, Department of Chemistry and Biochemistry, South Carolina, USA

Six papers on AGE breakers have been catalogued in PubMed during the last year. One of the most recent, by Cheng *et al.* (1), describes a novel AGE breaker, a benzoyl analog of the original compound, N-phenylacetyl-thiazolium bromide. The authors demonstrate that C36, does everything that AGE breakers are supposed to do: 1) release AGE-albumin from preformed AGE-albumin-collagen complexes; 2) release immunoglobulins from red cells of diabetic rats; 3) decrease crosslinking of collagen in diabetic rats; (4) reduced fluorescence in diabetic collagen; (5) improve several indices of cardiovascular performance in streptozotocin diabetic rats; and (6) reduce the expression of several diabetes-related genes, including RAGE, TGF β and connective tissue growth factor.

This study sounds convincing, based on the title and abstract. However, the experimental design is flawed. As described, a cohort of streptozotocin-induced diabetic rats is raised for 12 weeks without treatment, then divided into groups receiving either vehicle alone or ALT-711 or C36 for 4 additional weeks. All measurements are made at 16 weeks after induction of diabetes. Although all drug-treated diabetic rats had lower levels of AGEs (measured by collagen-linked fluorescence) and improved cardiovascular performance, compared to the untreated diabetic group at 16 weeks, there is no absolutely no evidence for AGE breaking, *i.e.* the entire effect could be attributable to inhibition of AGE formation between weeks 12 and 16, rather than AGE breaking. Some data on the untreated diabetic group at 12 weeks is essential to establish that the AGE breakers actually reduced AGEs in the 16 week diabetic rats to a level below that observed in diabetic animals at 12 weeks of age. In the absence of this information, there is no evidence for AGE breaking. Comparison of the effects of C36 and an AGE inhibitor might have strengthened the argument that C36 acted as an AGE breaker rather than an AGE inhibitor. In summary, although these studies support the role of AGEs in development of diabetic cardiovascular disease and suggest that C36 may have merit for treatment of diabetes, there is no evidence that C36 works by breaking pre-existing AGEs.

The fundamental problem with this study, and all other AGE breaker studies that have been published, is that none of them has ever demonstrated that AGE breakers actually break AGEs *in vivo*! The underlying problem is that, in more than a decade since the original description of the first AGE breaker, no one has identified a single AGE crosslink *in vivo* with vicinal carbonyl groups that might be cleavable by AGE breakers – despite the fact that about a dozen new AGEs have been identified since that time. With the techniques of modern mass spectrometry, these compounds should be readily identifiable by analysis of crosslinked peptides, with or without NaBH₄ reduction to stabilize possibly labile structures. The pure crosslinks should also be isolable. Arguments that they are destroyed during acid hydrolysis of protein are no longer relevant – there are well-established methods for exhaustive proteolytic hydrolysis of proteins, which are commonly used, for example, for analysis of glucosepane and methylglyoxal adducts in proteins.

As we have noted previously (2), AGE breakers are chemically unstable in aqueous solution, and their hydrolysis products are potent chelators, antioxidants and AGE inhibitors. Through these activities, they are likely to inhibit formation of AGEs and, by preventing new AGE formation during natural turnover of proteins in the extracellular matrix, they may mimic their putative AGE breaking activity through AGE inhibitory activity. It is also possible that they may discharge Schiff base or other aldehyde crosslinks and dicarbonyl adducts in proteins, including immature (natural) Schiff base crosslinks in collagen,

producing an apparent decrease in AGE crosslinks as a result of turnover of vascular collagen. Overall, while the study of Cheng *et al.* (1) provides support for the role of AGEs in diabetic complications, it fails to establish a mechanism of action dependent on AGE breaker activity. It is time to isolate the AGE that is a target for AGE breakers. These mythical compounds are presumably representative of a major, clinically significant class of AGEs, and presumably they are stable and abundant, considering their long-term impact in diabetes and aging. Until such time, why propagate the myth – there are no AGE breakers!

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N^{α} -(1-deoxy-D-fructos-1-yl)-L-histidine: A new breed of antioxidants in tomato powder?

Varoujan A. Yaylayan, *Department of Food Science & Agricultural Chemistry, Quebec, Canada*

Dried vegetable and fruits are considered a rich source of Amadori products they may constitute more than 10% of the water-soluble material per dry weight basis. Mossine and Mawhinney (2007) extracted the title compound from commercial tomato powder and measured its concentration using GLC-MS after a silylation step. The content of fructosyl-histidine was found to be 40mg/100g of dried tomato powder. This compound, afforded polymeric DNA nearly 100% protection from hydroxyl radical-mediated fragmentation in the presence of Cu/H₂O₂/ascorbate system. This antioxidant ability may be related to its specific metal binding capacity. Metal chelation may hinder availability of these redox-active metal ions to potential substrates and therefore prevent catalysis. Although free amino acids are known to chelate different metals in their +2 oxidation state, however, the fructosyl residue in the Amadori product may enhance the stability of the metal complex through additional bonding provided by the C-2 and C-3 hydroxyl groups as shown in Figure 1a.

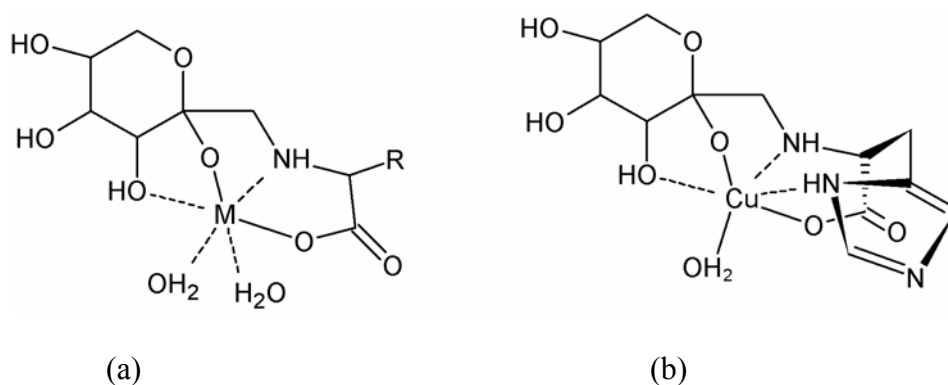


Figure 1. Metal complexes of Amadori products (a) general (b) Histidine

Copper binding ability of this compound was studied using potentiometric titration and compared with structurally similar molecules such as free histidine, carnosine, fructosyl-arginine. The stability constants calculated for the 1:1 complexes formed by fructosyl histidine and Cu(II) in neutral aqueous solutions was about 10^4 fold higher than the values calculated for histidine, carnosine and fructosyl arginine. In addition this complex was stable to redox changes in the pH range 2 - 10.5. The main coordination species of composition MLH₁ is shown in Figure 1(b). The authors propose to consider fructosyl histidine as a potential dietary antioxidant.

References

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A New CML Database

Monika Pischetsrieder, Department of Chemistry and Pharmacy, Erlangen, Germany

Among the advanced Maillard products, CML has been studied most intensively, both in medical and in food science. Many studies deal with reaction pathways leading to CML formation and its physiological and nutritional relevance. Furthermore, many methods have been established to analyze protein bound CML, including LCMS/MS, GCMS, ELISA and MALDI TOF MS. Consequently, solid data are available on the occurrence of CML *in vivo*. In contrast to this abundant information on CML, it is surprising that we have only little quantitative data on CML concentrations in various food products.

In a recent study by Assar et al., ultra performance liquid chromatography coupled to ESI MS/MS was applied to analyze CML in acid hydrolyzed food proteins after borohydride reduction¹. CML was quantified by isotope dilution analysis. The method is definitely state of art so that reliable data on the CML content in various food stuffs can be expected. Highest CML formation rate was determined in bread crust and evaporated whole milk with 15.2 and 4.86 mmol CML/mol Lys, whereas lowest levels were found in raw meat and raw or pasteurized milk (0.03, 0.08 and 0.09 mmol CML/mol Lys). CML was not detected in olive oil. Charissou et al. recently measured CML in some food products by GCMS, but it is difficult to compare these two data sets, because of differences in food products chosen for analysis and differences in the expression of the CML content (mg/kg protein or mmol/mol Lys)². However, it is obvious that the present data clearly differ from those of an often cited database on the CML contents in various food products³. In the latter study, highest CML concentrations were reported for olive oil and butter. The most likely explanation for this discrepancy is the interference of food matrix components with the ELISA assay used to generate this data base. This assumption is supported by a recent study from Buetler et al. who calculated a correlation of the CML content reported in the data base with the fat content of the food products⁴. It is tempting to conclude from this correlation that the interferent is the high fat content of some food products.

On the other hand, it must also be stated that in some well defined food systems, such as processed milk, CML ELISA can provide very reliable data which correlate well with data obtained by chromatographic – mass spectrometric methods^{Error! Bookmark not defined.}. Thus it can be concluded that the food matrix remains always a challenge -and sometimes a nightmare- for the analysts asking for stringent quality control of their methods. However, the

present study seems to be a good start to generate a new and reliable data base to survey CML concentrations in food products.

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CML – AGE or ALE?

John Baynes, Department of Chemistry and Biochemistry, South Carolina, USA

CML was one of the first AGEs to be identified and still remains a quantitatively important chemical modification of proteins in aging and disease. In 1996, however, Fu *et al.* (1) showed that CML was formed, not only from carbohydrates, but also from lipids during lipid peroxidation reactions. Januszewski *et al.* (2) subsequently proposed that CML was formed primarily as a result of lipoxidation reactions and suggested that chemical modification of proteins by lipids was more significant than chemical modification of proteins by glucose or other carbohydrates in diabetes. However, there was, until recently, little direct evidence on the relative importance of lipids vs. carbohydrates in formation of CML.

IMARS HighLights is designed to draw attention to important advances in research on the Maillard reaction. In this case, I would like to highlight a figure which is buried in the scientific literature: Figure 1 in Beutler *et al.* (3). In this figure (below), the authors provide some foundation for arguing that lipids are more important than carbohydrates in formation of CML. This conclusion is based on comparison of the CML content with the carbohydrate and lipid content of over 100 food products. The correlation coefficients between CML and the carbohydrate and lipid content of these foods were 0.053 ($p = 0.02$) and 0.53 ($p < 0.0001$), respectively. Notably, the correlation coefficients differ by a factor of 10 and the p -values by more than 100, supporting the authors' conclusion that "CML is more closely associated with the fat content and probably lipid peroxidation upon heating than with the Maillard reaction."

Strangely, these data and the conclusion regarding the source of CML are not mentioned in the Abstract or Discussion of the paper, nor does the title (3) provide a hint of their presence in the paper. Why? Probably because there is no experiment! The analyses of the CML content of foods are based on a study by Goldberg *et al.* (4) in which CML is measured by an ELISA assay; most of these data are available only in on-line supplements to the paper. The estimates of the carbohydrate and lipid content of foods are based on the Food and Nutrient Database for Dietary Studies 1.0, published by the United States Department of Agriculture. So how else could you publish a single, conceptually important, non-experiment today, except to bury it in an otherwise important study – in this case, one which shows convincingly that CML is not a ligand for RAGE – and hope that the reviewers and editors overlook the disconnect between the figure and the topic of the paper.

Despite its potential significance, there are several reasons to be concerned about the conclusions of this figure: 1) the analyses of the CML content are based on an ELISA, while the authors are fully capable of conducting more rigorous analyses of CML by LC-MS/MS, as documented elsewhere in the paper; 2) measurements of the total carbohydrate content of a food may be misleading because smaller reducing sugars (glucose, fructose, lactose) and ascorbate are more likely sources of the CML than are starches; 3) measurement of the total lipid content of foods may be misleading because polyunsaturated fatty acids are more significant sources of CML than is the total fat content; and 4) conditions for cooking of the various foods are not considered – fatty foods are likely to be cooked at higher temperatures for longer times than carbohydrate-rich foods. Thus, despite my personal bias that the authors are correct in their conclusions regarding the relative importance of fats vs. sugars in formation of CML, the experiment should be done correctly by measuring CML by chemical methods, by comparing the CML content of cooked foods with their reducing sugar and PUFA content, and by correcting for differences in cooking time and temperature of fatty vs. carbohydrate-rich foods. Comparison of milk products containing different amounts of fats

and processed under different conditions should be sufficient to make the point strongly. At the same time, it might be worthwhile to measure “Maillard-type fluorescence” in these samples; perhaps fluorescence is also derived primarily from lipids! The results of analysis of food products might provide some insight into the origin of CML and protein-linked fluorescence *in vivo*, since blood glucose and blood lipids tend to increase in concert with one another in diabetes and aging.

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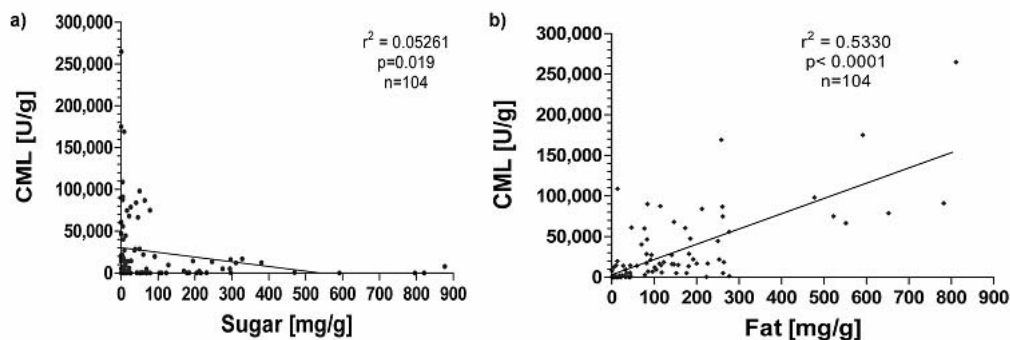
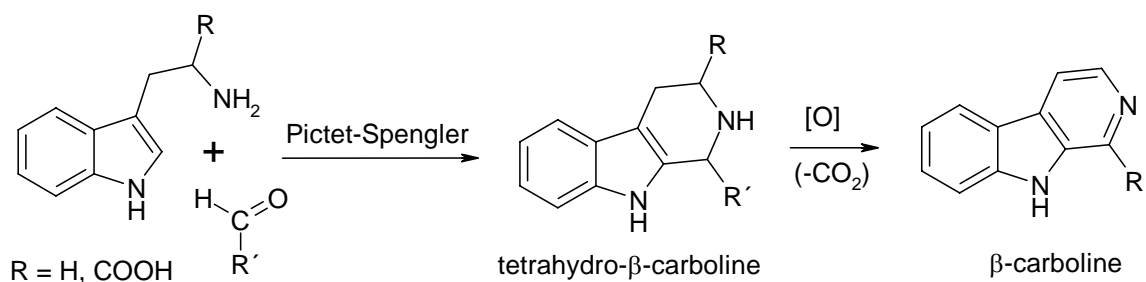


Figure 1. Correlation of CML content with sugar (A) and fat (B) content of 104 food items. CML data are from [10], the food sugar and fat content are from the Food and Nutrient Database for Dietary Studies 1.0 (<http://www.ars.usda.gov/Services/docs.htm?docid=7783>).

Mood Food

Monika Pischetsrieder, Department of Chemistry and Pharmacy, Erlangen, Germany

Whereas the physiological activity of many Maillard products is a matter of speculation, interesting biological effects of tetrahydro- β -carbolines and β -carbolines are well established. Tetrahydro- β -carbolines are formed by a Pictet-Spengler reaction between tryptophan or tryptamine on the one side and an aldehyde on the other side. Aldehydes, which take part in this reaction, are for example sugars or sugar degradation products. Tetrahydro- β -carbolines are then able to oxidize spontaneously yielding the corresponding β -carbolines.



Tetrahydro- β -carbolines and β -carbolines have interesting neuro-pharmacological activity. Harman and norharman, which are the β -carbolines derived from acetaldehyde and formaldehyde, respectively, inhibit for example monoamine oxidases, monoamine uptake and bind to the benzodiazepine binding site of the GABA receptor. Inhibition of monoamine oxidase and monoamine uptake increases the concentration of neurotransmitter, such as dopamine, in the synaptic cleft. Thus, monoamine oxidase and monoamine uptake are important drug targets for antidepressiva. Drugs, which act as agonists of the benzodiazepine binding site of the GABA receptor, on the other hand, are used as tranquilizers. The intake of harman has been further related to psychotropic effects of alcohol and tobacco smoke.

In several studies during the last years, mainly the group around Herraiz has shown that different tetrahydro- β -carbolines and β -carbolines are formed during food processing by the reaction of tryptophan with sugar degradation products¹. In more recent studies, it was shown by the same group that food derived β -carbolines are indeed able to inhibit monoamine oxidases *in vitro*^{2,3}. Thus it remains to speculate if dietary β -carbolines and tetrahydro- β -carbolines may cause mild neurotropic effects.

In this context, a new paper from Nemet and Varga-Defterdarović is of interest⁴. The authors synthesized a host of potential reaction products of tryptophan, tryptophan methylester and tryptamine with methylglyoxal. These reference compounds were then used to elucidate the major reaction products and pathways of the Pictet-Spengler reaction of methylglyoxal. The identification of reaction pathways leading to β -carbolines and tetrahydro- β -carbolines during food processing is important for the search for new potentially neuroactive derivatives. Furthermore, the development of synthetic procedures yielding sufficient quantities of pure test compounds promotes studies on their physiological properties *in vitro* and *in vivo*.

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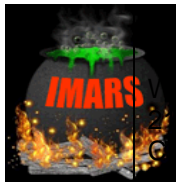
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Highlights of the glycation literature March– April 2008

The proceedings of “The Ninth International Symposium on the Maillard Reaction - Munich, September 1-5, 2007” The Maillard Reaction – Recent advances in Food and biomedical Sciences. Annals of The New York Academy of Sciences, Volume 1126.

The Junior Scientist Writing Competition

This is the first announcement for **The Junior Scientist Writing Competition**. Articles are invited for the competition mentioned above from junior scientists including PhD students, post-doctoral research fellows and other researchers who just started research in the glycation field. Deadline for the submission and further details will be published in coming Highlight issues. Winners will be announced at The Tenth International Symposium on the Maillard Reaction – Australia, September 2009












INTERNATIONAL MAILLARD REACTION SOCIETY

plstein Building
03 Cornell Road, Room 5127
Cleveland, Ohio 44106. USA.

Phone: 216-368-6613
Fax: 216-368-1357
E-mail: imars@case.edu



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