Toxicity of uraemia—does it come of AGE?

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Introduction

In the course of normal ageing, and more markedly during hyperglycaemia, proteins are covalently modified and cross-linked through the generation of a family of related adducts which form slowly and irreversibly on most body proteins. Reducing sugars, e.g. glucose, interact with free amino groups to yield Schiff bases; in the course of days these are rearranged to form Amadori products. Over weeks such reversible products are slowly transformed into irreversibly cross-linked advanced glycosylation end-products (AGE); furoyl-furanyl-imidazole (FFI) has been claimed to be one of the cross-linking multifunctional groups, although doubts about its existence in vivo and the possibility of isolation artefacts persist. The acronym AGE was specifically chosen to indicate that a relation exists to the normal ageing process [1]. The AGE reaction depends on the availability of reducing sugars; hence the formation of AGE occurs at a faster rate in patients with diabetes mellitus.

Formation of AGE through cross-linking is selectively inhibited by aminoguanidine, although this component is not a specific reagent and it participates in many reactions other than the Amadori reaction. While some complications of diabetes mellitus may be prevented by aminoguanidine, the specificity of this observation is uncertain. For the nephrologist it is interesting to consider whether endogenous guanidines accumulating in renal failure, e.g. methylguanidine, similarly interfere with cross-linking.

In patients with uraemia Flückiger et al. [2] documented carbamylation of haemoglobin; this resulted from interaction of urea-derived cyanate, with free amino-groups of the amino acids of haemoglobins (as well as other proteins). Such monofunctional carbamylated derivatives, however, do not form cross-linked advanced products—in contrast to the glycosylated derivatives.

Why are AGE important?

AGE modification of protein appears to be an important mechanism to tag senescent proteins and to provide a recognition signal for their removal. They interact specifically with a receptor (RAGE) or receptors on endothelial cells and macrophages which are distinct from other scavenger receptors, e.g. from the receptor for acetylated LDL, malonylated albumin or the mannose/fucose receptor. One RAGE has recently been cloned and characterized [3]. Formation of AGE not only triggers removal of the modified proteins, but also stimulates formation of cytokines, e.g. TNF and IL-1, and growth factors, e.g. IGF-1 and PDGF. These factors are involved in cellular growth and matrix formation. The final result of removal and growth is remodelling of senescent structures. AGE have not only been related to disturbed endothelial permeability (because of reduced proteoglycan binding, abnormal degradation, or abnormal self-assembly of membrane constituents), but also to vessel-wall thickening (because of resistance of AGE products to proteolysis; trapping of plasma proteins through AGE-mediated cross-linking; cell proliferation). Finally AGE cause reduced vasorelaxation through quenching of nitric oxide (NO) [4]. It is of interest to note that resistance to NO is also a feature of uraemia. Finally AGE interfere with the antiproliferative action of NO.

Why may this be important for renal failure?

The receptor for AGE (RAGE) is expressed on a macrophage tumour cell line (RAW-264.1). This permitted establishment of a competitive radioreceptor binding inhibition assay. In a first step bovine serum albumin (BSA) is transformed to AGE products (AGE–BSA) and radiolabelled by preincubation with radioactive glucose phosphate. If AGE peptides are present in the sample to be analysed, specific binding of such prelabelled AGE–BSA to RAW-264.1 cells is inhibited. Using this technique, Makita et al. [5] found increased AGE peptides, mostly in the molecular weight range less than 10 kDa in diabetic patients without renal failure. Their concentration was even greater in non-diabetic patients on dialysis, and greatest of all in diabetic patients on dialysis. The following observations suggest that AGE peptides accumulate as a result of impaired renal function: a significant relation existed between AGE peptides and serum creatinine;
Furthermore, their concentration decreased rapidly after renal transplantation. More recently, a competitive ELISA has been established which showed that the serum concentration of AGE peptides was decreased by high-flux dialysis, but not by conventional dialysis or CAPD [6].

Are glucose-derived furanyl-furanyl-imidazole (FFI) end-products the only AGE that accumulate in renal failure?

When analysing skin collagen, Sell and Monnier [7] surprisingly noted covalent cross-linking of protein by interaction with a pentose (instead of a hexose). This product, named pentosidine, was noted in diabetic patients, in non-diabetic patients with renal failure, and particularly in diabetic patients with renal failure. Even in healthy subjects it increased as a function of age. Such fluorescent pentosidines, i.e. peptides cross-linked with pentoses, could be synthesized in vitro from L-lysine/L-arginine polypeptides and a number of pentoses, e.g. D-ribose, xylose, arabinose, and lyxose. More recently it could be shown that pentoses are not the only precursor, since pentosidines could also be synthesized from glucose, Amadori products, fructose, and even dehydro-L-ascorbate. Although the in-vivo precursor has not been identified, it is of note that elevated plasma pentosidines were found in diabetic subjects (2.5-fold elevation) and particularly in uraemic diabetic subjects (23-fold elevation) [8].

These recent findings potentially open new windows for the understanding of some specific problems of uraemia, e.g. the excessive prevalence of vascular problems, presence of capillary basal membrane thickening, diminishing resilience of extracellular matrix ('shrinking man syndrome'), and macrophage dysfunction, to mention only a few.

In another context, AGE peptides may represent a modern-day version of 'middle molecules'. If they were pathophysiologically relevant, their presence would provide a strong rationale for the use of high-flux membranes.

Before such considerations are entertained, however, some critical questions must be answered:

- Are circulating AGE peptides pathogenic?
- Is it worthwhile to remove them?
- Do products such as FFI or pentosidine interact in vivo with monocyte receptors of uraemic patients?
- Are low molecular-weight AGE peptides deposited in extravascular tissues?
- What types of AGE are important? FFI, pentosidines, both types or others?
- Do they contribute to the pathogenesis of atheroma, stiffening of connective tissue, and the accelerated ageing of dialysed patients?
- And if 'yes', can this outcome be prevented by their removal, reduced generation and/or manipulation of their receptors?

It is certain that resolution of these questions will keep the dialysis community busy for years to come.

References