PHARMACOLOGICAL MODULATION OF AGEs: A UNIQUE ROLE FOR REDOX-ACTIVE METAL IONS

Miriam F. Weiss,1 Amit K. Saxena,2 Vincent M. Monnier2

Division of Nephrology; Department of Internal Medicine, University Hospitals of Cleveland, 1 and Institute of Pathology and Department of Biochemistry; Case Western Reserve University

School of Medicine2

Pharmacological modulation of the effects of advanced glycosylation end-products (AGEs) in patients with end-stage renal disease (ESRD) can be approached using three major strategies: (1) suppression or prevention of formation, (2) reversal of crosslinks already present on proteins, and (3) inhibition or blockade of the biologic effects of AGEs. This paper will provide a brief review of the literature supporting each of these approaches, and will focus on a newly defined mechanism for accelerated AGE formation and toxicity in ESRD. This novel mechanism, involving redox-active metal binding to proteins, may provide the basis for a unique and simple approach to pharmacologic modulation of AGE effects in ESRD.

STRATEGIES FOR PREVENTION OF AGE FORMATION

Glucose and its auto-oxidation products, Amadori degradation products, reactive carbonyl fragments, and increased oxidative stress, all contribute to the formation of AGEs. The relative importance of each mechanism has not been defined in ESRD. Nonetheless, models based on approaches to inhibiting specific mechanisms have progressed from in vitro experiments to clinical trials in some cases.

REDUCE LEVELS OF REACTIVE PRECURSORS

The importance of controlling hyperglycemia in the prevention of diabetic complications has been well documented (1). In ESRD, the presence of the diabetic state has no apparent effect on AGE levels (2-4).

KEY WORDS: Modulation of AGEs; advanced glycosylation end-products.

Nonetheless, adequate control of hyperglycemia in patients with diabetes and ESRD may reduce morbid complications (5). The correlation between increased dose of dialysis and circulating levels of AGEs suggests that dialysis removes a low molecular weight reactive precursor (4). However the identity of that precursor remains unclear.

Recent attention has focused on the role of glucose degradation products in commercially available peritoneal dialysate (6). Increased levels of 3-deoxyglucosone (7), and glyoxal, methylglyoxal, and other aldehydes (8) are present in dialysate manufactured by heat sterilization, but not by filter sterilization. AGE formation is slowed in icodextrin dialysate solutions compared to glucose dialysate solutions (9), and in two-compartment bags in which the glucose and electrolyte solutions are heat-sterilized separately (10). Thus improvements in the manufacture of peritoneal dialysate may result in less glycation of the peritoneal membrane, with potential for improving technique survival (6).

SCAVENGE OR BLOCK THE EFFECT OF REACTIVE CARBONYLS

Aminoguanidine: The mechanism of action of aminoguanidine is not completely clear. In vitro, this agent reacts with Amadori-derived products such as 3-deoxyglucosone, and dicarbonyls such as glyxal and methylglyoxal, to prevent subsequent AGE formation on proteins. It may also prevent protein cross-linking by Amadori product already bound to protein (11). In animal models of diabetes, aminoguanidine has been shown to prevent diabetes-induced arterial wall protein cross-linking (12), and to inhibit the development of diabetic retinopathy (13). Aminoguanidine is also an inhibitor of nitric oxide synthase and may ameliorate diabetic vascular dysfunction through reduction of NO production, in addition to its effect on AGE formation (14). Trials in patients with ESRD are currently underway (15).
Because the agent has both antioxidant (at high dose) and pro-oxidant (at low dose) activity towards low density lipoprotein (LDL) (16), its therapeutic benefit remains in question. Newer agents, such as the thiazolidine derivative OPB-9195, block both glycoxidation and lipoxidation reactions in animal models of diabetes (17).

Enhance Antioxidant Defenses: The final common pathway for antioxidant benefit against AGE formation may be through increasing the amount of both intracellular and extracellular reduced glutathione (GSH). GSH is a free-radical scavenger that detoxifies peroxides and free radicals (18) and acts as a cofactor for enzymes that detoxify reactive dicarboxyls (19). Alpha-tocopherol improves GSH levels, decreases superoxide production, and decreases the reactive dicarbonyls (19). Alpha-tocopherol improves GSH (18) and acts as a cofactor for enzymes that detoxify extracellular reduced glutathione (GSH). GSH is a free-radical scavenger of reactive oxygen species to normal (35). The agents reviewed above show therapeutic promise. Equally important is their utility in elucidating mechanisms leading to AGE formation and toxicity. Unfortunately for nephrologists, most of the literature in this field is based on models of diabetes in which the mechanisms of formation and the toxic role of AGEs may differ significantly from that occurring in ESRD. The remainder of this paper will focus on a novel approach to reversing AGE toxicity in ESRD.

STRATEGIES TO BREAK THE VICIOUS CYCLE OF AGE-INDUCED OXIDATIVE STRESS

Depletion of Reactive AGEs from Serum Using a Lysozyme-Linked Matrix: Lysozyme enhances the uptake and degradation of AGE-rich proteins by macrophages. Therefore lysozyme bound to a gel matrix was used to strip AGE-rich proteins (defined by ELISA) from the serum of patients with ESRD. The AGE-rich proteins that bind to the lysozymelinked matrix are relatively high molecular weight (HMW), including IgG, complement components such as C3b, and apolipoprotein J (ApoJ) (36). Based on these observations, a "lysozyme dialyzer" is being considered for clinical studies.

At present, the clinical effectiveness of this approach is difficult to assess. More than 95% of structurally identified serum AGEs are present on the high molecular weight fraction of serum (2,37). No current evidence exists that specific HMW proteins (such as IgG, C3b, and ApoJ) contain proportionally greater quantities of AGEs than albumin. However, these proteins may have enhanced toxicity owing to increased binding to specific receptors on cells, thereby inducing cellular oxidant stress in a vicious cycle that contributes to increased rates of AGE formation.

REDOX-ACTIVE METAL BINDING BY THE AGE

Nε-(CARBOXYMETHYL)LYSINE

Biologic Effects of Redox-Active Metals: A longstanding controversy in the biology of free radicals is whether the reactions catalyzed by transition metals in the test tube can occur in vivo (38). In health, metal ions are tightly sequestered by binding proteins such
as ferritin, transferrin, and ceruloplasmin. These binding proteins reduce the availability of free ions to serve as radical generators in Fenton-type reactions (39). Albumin is a particularly important extracellular antioxidant, binding metal ions in vitro as well as in vivo (40). Even in illnesses characterized by iron overload, reactive iron species cannot be found in blood or tissues (39).

Effect of Chelation on AGE Formation in Uremic Serum and Spent Peritoneal Dialysate: Pentosidine forms at an increased rate in serum from patients treated by HD and PD compared to serum from normal controls (4). Incubation of proteins in 1 mol/L glucose (although 100-fold physiologic) is widely accepted as a way to uncover the effects of redox-active transition metal catalyzed oxidation reactions (41,42). Therefore normal serum, serum from patients treated by HD, and spent peritoneal dialysate to which 65 mg/mL of normal human serum albumin (HSA) had been added were incubated in the presence of 1 mol/L glucose for up to 20 days (Figure 1). Chelating agents DTPA (diethylene triaminopentaacetate) (1 mmol/L) and phytic acid (1 mmol/L) were the most effective suppressors of the formation of pentosidine in serum from patients on HD. They were also more effective than all others tested in serum from healthy controls. The effect of chelators was compared with that of: heat inactivation (to destroy enzymatic activity), aminoguanidine (5 mmol/L) -to block Amadori product formation), or catalase (100 U daily -to block H2O2 production) (43). In addition, chelators had a profound inhibitory effect on Nε-(carboxymethyl)lysine (CML) formation in uremic fluids (both serum from HD and spent peritoneal dialysate), but not in healthy serum. These results suggest that redoxactive metals are involved in the formation of AGEs in uremic fluids.

Nε-(Carboxymethyl)lysine as a Metal Chelator: Glycine is a known chelator of copper and other metals (44). Because CML and EDTA have a glycine-like configuration, it is conceivable that two appropriately configured CML residues on proteins can create a coordination sphere that can bind metals. To test this hypothesis, bovine serum albumin (BSA) was reductively alkylated with glyoxylic acid at various concentrations to induce the formation of CML. The CML-modified protein was exposed to 500 μmol/L CuCl2, and dialyzed extensively to remove unbound metal. Cu2+ was then quantitated by atomic absorption, and by its ability to oxidize ascorbic acid to dihydroascorbate. The quantity of CML bound to BSA correlated with increased binding of Cu2+ and a concomitant increase in the oxidation of ascorbate (45).

The biological relevance of CML-protein-metal complexes in patients with ESRD is likely to depend on several factors: the extent and site of specificity of CML formation on proteins, the availability of metal ions for binding to form CML-protein-metal complexes, and the ability of CML-protein-metal complexes to undergo redox cycling. To demonstrate the biological relevance of these observations, CML-rich proteins in the serum of normal subjects and patients with ESRD were immunoprecipitated using a highly specific anti-CML polyclonal antibody. The resultant CML-rich fraction demonstrates a markedly increased ability to oxidize ascorbate. This increased ascorbate oxidation can be normalized by the addition of chelating agents (Table 1). Increased redox activity of immunoprecipitated CML-rich proteins from patients with ESRD is also demonstrated by electron spin resonance analysis. In summary, these data demonstrate that the major AGE product of the Maillard reaction, CML, has metal-binding.

Figure 1 — The pattern of increase in the content of pentosidine in hemodialysis (closed symbols) and healthy (open symbols) serum (A). Effect of inhibitors on CML formation in healthy serum (B), hemodialysis serum (C), and pooled spent peritoneal dialysate (D). Key to inhibitors as in panel B.
properties. Therefore, AGEs may induce "uremic toxicity" through a unique mechanism, the formation of metal coordination spheres that can catalyze increased oxidative stress.

CONCLUSIONS AND FUTURE DIRECTIONS

The mechanism of formation of the major structurally identified AGE, CML, probably varies depending on the tissue site and the disease process. In collagen-rich tissues, and in the presence of diabetes, CML most likely originates from hydroxyl radical-mediated fragmentation of the Amadori product present on glycated proteins (46). However, glyoxal/glycolaldehyde are the common precursors of lipid peroxidation and myeloperoxidase-catalyzed serine oxidation (47). Thus one can imagine a vicious cycle, initially consisting of the accumulation of AGEs with renal failure, followed by transition metal binding and hydroxyl radical generation in the presence of H2O2. In lipid-rich tissues, or at sites of inflammation, lipid peroxidation would further enhance this process.

In conclusion, we envision that future pharmacological manipulation of AGE formation will involve chelation therapy. Chelation in patients with ESRD is not new. Desferoxamine treatment of aluminum overload has been administered safely and successfully either intravenously during the HD treatment (48) or into the peritoneal dialysate (49). To avoid potential long-term toxicity, a cartridge with immobilized desferoxamine has been developed which can be used in series with traditional HD methods (50). We hope soon to demonstrate that the use of chelation therapy in patients with ESRD will break the vicious cycle of ever-accelerating AGE accumulation and formation, with resultant reduction in "uremic toxicity."

ACKNOWLEDGMENTS

Supported by DK-45619 (MF) and AG05601 (VM) from the National Institutes of Health.

---

**REFERENCES**


11. Edelstein D, Brownlee M. Mechanistic studies of ad


Ne-(Carboxymethyl)lysine, a molecular sink of protein aging by Maillard reaction, lipid peroxidation and inflammation, coordinates divalent and redox-active metals. *Science.* 1998; [In press].


48. Barata J, D'Haese P, Pires C, Lamberts L, Simoes J,