Chelation: A Fundamental Mechanism of Action of AGE Inhibitors, AGE Breakers, and Other Inhibitors of Diabetes Complications

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This article outlines evidence that advanced glycation end product (AGE) inhibitors and breakers act primarily as chelators, inhibiting metal-catalyzed oxidation reactions that catalyze AGE formation. We then present evidence that chelation is the most likely mechanism by which ACE inhibitors, angiotensin receptor blockers, and aldose reductase inhibitors inhibit AGE formation in diabetes. Finally, we note several recent studies demonstrating therapeutic benefits of chelators for diabetic cardiovascular and renal disease. We conclude that chronic, low-dose chelation therapy deserves serious consideration as a clinical tool for prevention and treatment of diabetes complications. 

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AGE HYPOTHESIS

The Maillard or advanced glycation end product (AGE) hypothesis on the pathogenesis of diabetic vascular complications proposes that increased protein glycation during hyperglycemia and accelerated accumulation of AGEs on long-lived tissue proteins are fundamental processes underlying the development of diabetes complications (1,2). The terms “autoxidative glycosylation” and “glycoxidation” (3) were introduced at an early stage in this research field to highlight the importance of oxidation chemistry in AGE formation. Reactive oxygen species (ROS) and free (decompartmentalized) metal ions were identified as key participants in the Maillard reaction, and chelators were identified as potent inhibitors of browning and cross-linking of proteins by glucose. Oxygen was described as a fixative of irreversible damage to proteins via the Maillard reaction, and today metal-catalyzed oxidation reactions and chemical modifications of proteins, including numerous AGEs (Fig. 1), advanced lipoxidation end products (ALEs), and protein oxidation products, are implicated in many chronic diseases involving oxidative stress, including diabetes and cardiovascular and neurodegenerative diseases (1–5).

AGE INHIBITORS

Aminoguanidine. In 1986, Brownlee et al. (6) introduced the first AGE inhibitor, aminoguanidine, as a trap or scavenger of reactive carbonyl intermediates in the Maillard reaction (Fig. 1). In numerous studies in animal models of both type 1 and type 2 diabetes, aminoguanidine inhibited AGE formation in concert with inhibition of diabetic renal, retinal, neural, and vascular complications (7). Aminoguanidine is administered at a relatively high dose (typically 1 g/L in drinking water); in severely hyperglycemic rodents, which may consume their body weight in drinking water per day, this dose is equivalent to ~1 g/kg/day. While the dose is enormous, it is not unreasonable; aminoguanidine has a short plasma half-life (~1 h), and AGE inhibitors must be present at a concentration sufficient to continuously react with and trap chemical intermediates in the Maillard reaction (Fig. 1). High aminoguanidine concentrations are required to drive sluggish and thermodynamically unfavorable trapping reactions to completion. More reactive carbonyl traps are likely to be toxic, e.g., because of their reaction with and depletion of vitamin B6, pyridoxal. While aminoguanidine is the prototype AGE inhibitor, its proposed mechanism of action is based completely on model chemical studies in vitro. Today, >25 years since its discovery, there is no published evidence that aminoguanidine traps AGE precursors in vivo; i.e., none of the types of adducts described in Fig. 1 have been detected in urine or plasma.

Pyridoxamine. The B6 vitamer pyridoxamine was described as an Amadorin or post-Amadori AGE inhibitor, trapping products derived from the Amadori compound fructoselysine, the first stable glucose adduct to protein (8). Pyridoxamine is now considered to have multiple mechanisms of action: 1) blocking oxidation of the Amadori intermediate; 2) trapping of reactive carbonyl and dicyranyl compounds derived from the Amadori compound; 3) chelation of metal ion catalysts of oxidation chemistry; and 4) scavenging of ROS (9). As observed with aminoguanidine, pyridoxamine inhibited the full range of diabetic vascular complications in animal models of both type 1 and type 2 diabetes (8,9). In addition to their AGE-inhibitory activity, both aminoguanidine and pyridoxamine significantly lowered plasma triglycerides and cholesterol (10). Although pyridoxamine adducts of lipid peroxidation products have been identified in animal models of diabetes (11), like aminoguanidine, not a single product of reaction of pyridoxamine with a dicarbonyl intermediate in AGE formation, such as methylglyoxal or deoxyglucosones, has been identified in biological systems. Considering the sensitivity and specificity of modern mass spectrometric techniques, it seems unlikely that this is the result of technical limitations.
Other carbonyl-trapping agents. A number of other compounds with reactive nucleophilic functional groups and carbonyl-trapping activity in vitro are effective as AGE inhibitors in rodent models of diabetes, including 2,3-diaminophenazine, OPB-9195, tenilsetam, penicillamine, and several derivatives of aminoguanidine (Fig. 2A) (4,5,12). However, like aminoguanidine and pyridoxamine, no products of reaction of these compounds with intermediates in glycation or glycoxidation reactions have been identified in vivo. Although penicillamine, carnosine, and possibly lipoic acid have carbonyl-trapping activity in vitro, their potent chelating activity, measured by inhibition of metal-catalyzed ascorbate oxidation (13), would contribute to their AGE-inhibitory activity. To the best of our knowledge, analogs of these compounds with AGE-inhibitory activity, but lacking in chelating activity, have not been identified.

Thiamine and benfotiamine. Thiamine and benfotiamine are often described as AGE inhibitors but have a much broader spectrum of action. In a unifying hypothesis on the mechanism of diabetes complications (14,15), Brownlee (14) and Giacco and Brownlee (15) proposed that inhibition of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by mitochondrial-derived ROS is a pivotal step in the pathogenesis of diabetes complications. The resultant accumulation of glycolytic intermediates leads to activation of the polyol, hexosamine, and diacylglycerol–protein kinase C pathways and increased formation of methylglyoxal-derived AGEs and expression of the receptor for AGE (RAGE). Thiamine and benfotiamine correct these metabolic changes, it is proposed, by diverting glucose metabolism from glycolysis through activation of transketolase, the thiamine-dependent enzyme in the pentose phosphate pathway (PPP) (16). Indeed, benfotiamine reverses all of the observed metabolic changes induced by hyperglycemia in vitro, including formation of methylglyoxal-derived AGEs; it also inhibits complications in diabetic animals and has shown promise in clinical trials (16). Transgenic overexpression of superoxide dismutase, which would limit ROS-dependent inactivation of GAPDH, also reversed many of the metabolic changes, as well as AGE formation, and inhibited development of complications in diabetic mice (15).

Studies with thiamine and benfotiamine illustrate that alterations in enzymatic pathways in diabetes may have a significant impact on oxidative stress and formation of AGEs. Glyceraldehyde-3-phosphate, a major precursor of methylglyoxal, is one product increased as a result of inhibition of GAPDH by mitochondrial-derived ROS. Glyceraldehyde-3-phosphate is not just a source of methylglyoxal AGEs but is also among the most readily autooxidized sugars and thereby a source of superoxide for metal-catalyzed Fenton and Haber-Weiss reactions, leading to increased production of hydroxyl radicals. Effects on the concentration of hexose phosphates would explain reported effects of thiamine and benfotiamine on formation of other AGEs. However, benfotiamine also has anti-inflammatory effects in diseases other than diabetes (16), e.g., endotoxemia, suggesting that its mechanism of action is not limited to activation of the PPP or other thiamine-dependent pathways. There are, to our knowledge, no reports on the chelating activity of thiamine or benfotiamine, but both thiamine, in the form of thiamine diphosphate, and benfotiamine, because of its complex multidentate structure, are likely to be effective in chelation of metal ions.
Lalezari-Rahbar compounds and the effects of chelation. Lalezari-Rahbar (LR) compounds (named after the developers) are a new class of AGE inhibitors, a diverse group of aromatic organic acids with ureido and carboxamide functional groups (Fig. 2B) (17,18). These compounds have been shown to protect against complications in animal models of diabetes and to have profound lipid-lowering effects (19,20), mimicking the effects of aminoguanidine and pyridoxamine in diabetic rats (10). The common effects of the AGE inhibitors on AGEs and plasma lipids suggest similar underlying mechanisms of action. Yet, the LR compounds lack nucleophilic groups and do not trap carbonyl compounds under physiological conditions, even in vitro. Regardless, they are potent inhibitors of AGE formation in diabetic rats and mice at doses of 50 mg/L in drinking water, i.e., at ≤5% of the molar dose commonly used for aminoguanidine and pyridoxamine. The LR compounds appear to inhibit AGE formation primarily through their chelating activity; they are potent inhibitors of metal-catalyzed ascorbate oxidation, with half-maximal inhibition (IC_{50}) observed in the micromolar (micromole per liter) range (Table 1). Another recently described class of AGE inhibitors, derivatives of edaravone, has also been shown to have poor carbonyl-trapping activity but potent AGE-inhibitory activity (21). The IC_{50} for inhibition of ascorbate oxidation by TM2002, the most effective of these compounds, was 84 μmol/L (Table 1).

The LR compounds and edaravone derivatives have provided novel insight into the mechanism of action of AGE inhibitors. They suggest that chelation alone, independent of carbonyl trapping, is sufficient to inhibit AGE formation and protect against diabetes complications. Chelators would not only inhibit antioxidative glycosylation and glycoxidation but also inhibit enzymatic and metal-catalyzed ROS production after ligation of AGEs with scavenger receptors, such as RAGE. Thus, LR-90 inhibits the inflammatory response (increased expression of RAGE, monocyte chemoattractant protein-1, cyclooxygenase-2, and NADPH oxidase) in monocytes stimulated with the RAGE ligand, S100b, and also blocks the increase in monocyte endothelial cell adhesion (22). LR-90 also inhibited nuclear factor-κB activation after activation of the inflammatory response by tumor necrosis factor-α, suggesting that AGE inhibitors may exert wide-ranging effects on oxidative stress and inflammation by mechanisms other than carbonyl trapping.

Chelation is a common, but commonly overlooked, characteristic of most drugs with multiple functional groups. The chelating activity of AGE inhibitors varies widely: aminoguanidine and pyridoxamine inhibit ascorbate oxidation with IC_{50} in the 1–5 μmol/L range, while carnosine, dianmophenazone, OPB-3195, and tenilsetam are effective in the 5–50 μmol/L range (Table 1) (23). Though rigorous comparisons have not been conducted, the weaker the chelator, e.g., aminoguanidine or pyridoxamine, the higher the concentration generally used to inhibit the Maillard reaction in vitro and, in general, the higher the dose required to achieve efficacy in vivo. Even a weak chelator, however, in sufficient amount may deplete free or weakly bound metal ions by promoting their excretion in urine or bile.

ACE INHIBITORS, ANGIOTENSIN RECEPTOR BLOCKERS, AND OTHER ANTIHYPERTENSIVE AGENTS

ACE inhibitors are not the only drugs that inhibit AGE formation. Miyata et al. (24,25) demonstrated that ACE inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) inhibit the formation of AGEs in Maillard reactions in vitro. These compounds were poor carbonyl traps and were proposed to act primarily by inhibiting the oxidative formation, rather than by trapping, of reactive carbonyl intermediates. Several of the ACEIs and ARBs were shown to be potent inhibitors of metal-catalyzed oxidation of ascorbate, with IC_{50} generally in the 1–10 μmol/L range (Table 1). Forbes et al. (26) demonstrated that the ACEI ramipril, at ~10 μmol/L in drinking water (~0.1% the dose of aminoguanidine), was comparable with aminoguanidine in inhibiting renal AGE formation and albuminuria in diabetic rats. In these studies, renal nitrotyrosine was also decreased in both ramipril- and aminoguanidine-treated animals, suggesting that both compounds inhibit AGE formation, in part, through effects on ROS production. Liu et al. (27) showed that renoprotection by the ACEI benazepril in a spontaneously hypertensive (non diabetic) rat model was also accompanied by a decrease in AGE accumulation and expression of RAGE in the kidney, as well as decreased expression of NADPH oxidase p47phox and other biomarkers of oxidative stress. Monacelli et al. (28) reported that the ARB valsartan also decreased plasma and urinary AGEs in type 2 diabetic patients.

The relationship between the AGE-inhibitory and chelating activities of ACEIs and ARBs has not been systematically studied. The IC_{50} for ascorbate oxidation is 110 μmol/L for the ACEI temocaprilat (24) and <10 μmol/L for AVE8048, the active metabolite of the combination ACEI/vasopeptidase inhibitor AVE7688, which was a more potent AGE inhibitor (29). Izhara et al. (30) evaluated more than 100 ARB derivatives and focused on the compound R147176, which had potent AGE-inhibitory activity in vitro and also a low (~3 μmol/L) IC_{50} for ascorbate oxidation. Although R147176 had low affinity for the angiotensin receptor 1 and had minimal blood pressure-lowering activity, it was an effective inhibitor of nephropathy in three different rat models of hypertension and/or diabetes. R147176 has a core structure common to several ARBs, suggesting that the benefits of many ARBs are at least partly the result of their chelating and antioxidant activity (Table 1). Nagaku et al. (31) reported that the antihypertensive agent hydralazine, which does not interact with the renin-angiotensin system, also protected against nephropathy in the diabetic rat and was a potent AGE inhibitor both in vitro and in vivo; hydralazine has an IC_{50} for ascorbate oxidation ~1 μmol/L (31). In their comparison of the ARB olmesartan (IC_{50} ~5 μmol/L) with hydralazine, the authors noted peak plasma concentration of ~10 μmol/L for both drugs in clinical studies, indicating that tissue concentrations of the drugs should be sufficient to inhibit metal-catalyzed oxidation and glycoxidation reactions in vivo.

Miyata et al. (32) have summarized the evidence that antihypertensive agents have a range of renoprotective effects, independent of their blood pressure-lowering activity. Prominent among these effects is inhibition of oxidative stress, resulting in 1) decreased AGE formation, 2) decreased renal iron deposition, 3) decreased RAGE expression, and 4) decreased infiltration of inflammatory cells. All of these observations are consistent with the chelating and antioxidant activity of antihypertensive agents, which would limit both formation of AGEs and activation of AGE-RAGE-mediated inflammatory signaling cascades. The fact that ACEIs and ARBs are so effective in inhibition of AGE formation may explain in part why AGE inhibitors, despite
their introduction over 25 years ago (6), have not found use in clinical management of diabetes complications: they may provide little added protection in patients already being treated with ACEIs and ARBs.

**AGE BREAKERS**
The original AGE breaker, N-phenacyltiazolium bromide (PTB) (33), its dimethylthiazolium analog alagebrium (ALT)-711 (34), and recently described pyridinium analogs

**FIG. 2.** Structures of AGE inhibitors. A: First-generation AGE inhibitors. B: Novel LR compounds (adapted from Rahbar and Figarola [18]).
TRC4186 and TRC4149 (35,36) (Fig. 3) were designed to cleave AGE cross-links in tissue proteins. As support for their mechanism of action, these compounds 1) released AGE albumin from preformed AGE-albumin-collagen complexes, 2) released immunoglobulins bound to red cells of diabetic rats, and 3) reversed or decreased collagen cross-linking in diabetic rats. Despite these observations, not a single AGE cross-link structure identified in tissue proteins to date (Fig. 4) contains a dicarbonyl structure susceptible to the proposed mechanism of action of AGE breakers (Fig. 3). Indeed, the proposed target of AGE breakers, dicarbonyl cross-links, would be reactive carbonyl compounds, which are unlikely to accumulate in protein. Regardless of their target or mechanism of action, AGE breakers increase vascular elasticity and improve cardiovascular function in aging humans, in animal models of diabetes, and in other diseases (5,34,35,37).
Despite the lack of evidence for their AGE-breaking activity, AGE breakers are potent chelators, and their hydrolysis products have even stronger chelating activity than the intact compounds (23) (Table 1). In addition, all of the activities ascribed to AGE breakers in vitro assays, including dissociation of AGE-albumin complexes and immunoglobulin adducts on red cells, and in vivo effects on collagen cross-linking have been replicated in detail with several LR compounds (17,18), which lack functional groups that could cleave dicarbonyl compounds. In summary, despite several animal model and clinical studies that convincing demonstrate the merits of AGE breakers for treatment of cardiovascular and renal pathology in diabetes and aging (4,5,34–37), the effects of these compounds are unlikely to be related to their proposed mechanism of action and may be explained largely, if not completely, by their chelating and antioxidant activities. Chelation, by inhibition of glyoxidation reactions, would limit the progression from AGE precursor to AGE cross-link, enabling the rejuvenation of the extracellular matrix by turnover of cross-linked proteins and biosynthesis of native matrix proteins.

### ALDOSE REDUCTASE INHIBITORS

Aldose reductase inhibitors (ARIs) are designed to block excessive metabolism of glucose through the sorbitol pathway, thereby protecting against accumulation of fructose, dicarbonyl intermediates, and imbalances in NADPH- and glutathione-dependent antioxidant defenses. ARIs have a diverse range of structures, with side effects often attributable to their lack of inhibitor specificity (38). While their chelating activity has not been investigated systematically, all of them contain functional groups with potential chelating activity, including carboxyl, amino, imino, imidazole, oxo, thio, and hydroxy groups. Wolff and colleagues showed that ARIs inhibited metal-catalyzed oxidation of ascorbate and lipids (40), and Nakamura et al. (41) reported that the protective effects of the ARI NZ-314, a trioximidazolidine derivative, against diabetic neuropathy were in large part mimicked by the copper chelator trientine (triethylenetetramine). There are also several reports that ARIs inhibit AGE formation in diabetes and aging (5,42). Although they may act by reducing the concentration of AGE precursors, secondary mechanisms of action, such as chelation, may be important in understanding the broader impacts of these compounds on the pathology of diabetes and aging.

### CHELATORS

Alterations in iron and copper homeostasis are a characteristic feature of diabetes, evidenced by deposition of iron and copper in heart, kidney, and other tissues (43,44). Underlying the alterations in copper homeostasis are defects in copper absorption, tissue distribution, and an increase in loosely bound (chelatable) copper in tissues (45–45). Copper (summarized in 45) reported that, compared with control subjects, type 2 diabetic patients had higher basal urinary copper excretion and a greater increase in urinary copper in response to oral administration of triethylenetetramine, a well-tolerated copper chelator used clinically for treatment of Wilson disease. Administration of triethylenetetramine for 6 months caused a significant ~25% reduction of left ventricular mass toward normal in type 2 diabetic patients compared with an increase of 3% in left

#### TABLE 1

| IC₅₀ of various compounds that inhibit metal-catalyzed oxidation of ascorbate* | IC₅₀ (µmol/L) | Reference |
|---|---|---|
| AGE inhibitors | | |
| Aminoguanidine | 1,000–4,600 | 17, 23, 24 |
| Pyridoxamine | 1,000–2,500 | 17, 23, 24 |
| Carnosine | 0.7–7 | 17, 23 |
| Diaminophenazine | 50 | 23 |
| Edaravone | 114 | 21 |
| Lipoic acid | 125 | 17 |
| OPB-9195 | 3–5 | 23, 24 |
| TM2002 | 84 | 21 |
| Tenilstatem | 25 | 23 |
| LR-50 | 275 | 17, 18 |
| LR-9, 20, 59, 74 | 50–200 | 17, 18 |
| AGE breakers | | |
| PTB | 10 | 23 |
| PTB hydrolysate | <2 | 23 |
| ALT-711 | 80 | 23 |
| ALT-711 hydrolysate | <2 | 23 |
| ACEIs | | |
| Temocaprilat | 110 | 24 |
| AVE8084 | <10 | 29 |
| ARBs | | |
| Losartan | 1 | 30 |
| Olmesartan | 5 | 24, 30 |
| R-147176 | 3 | 30 |
| Other | | |
| Hydralazine | 1 | 31 |
| Chelators | | |
| EDTA, TETA, penicillamine | <1 | — |

*Based on inhibition of metal-catalyzed oxidation of ascorbate in neutral phosphate buffer, as previously described by Buettner (ref. 13).
FIG. 3. Structures (top panel) and proposed mechanism of action (bottom panel) of AGE breakers. According to this scheme, the dinucleophilic AGE breaker adds across a dicarbonyl AGE cross-link, followed by an internal rearrangement to cleave the dicarbonyl bond, breaking the cross-link. Following hydrolysis, the AGE breaker is regenerated, leaving a chemically inert CML on one peptide and a chemically reactive aldehyde functional group on the other peptide involved in the cross-link. (Adapted from Vasan et al. [33].)
FIG. 4. Structures of AGE cross-links identified in tissue proteins. Lys-Arg cross-links are shown in the *top panel* and Lys-Lys cross-links in the *lower panel*. Compounds with two or three carbon cross-links, e.g., GODIC, MODIC, GOLD, MOLD, and K2P, may be derived from both carbohydrates and lipids, i.e., they are AGE/ALEs. All of these compounds are considered to be irreversible AGE cross-links in proteins. Pentosidin, the vesperlysines, crosslines, and fluorolink are fluorescent and contribute to the increase in yellow-brown color and fluorescence of collagen in diabetes and aging. GODIC, glyoxal-derived imidazolium cross-link; MODIC, methylglyoxal-derived imidazolium cross-link; GOLD, glyoxal-lysine dimer; MOLD, methylglyoxal-lysine dimer.
ventricular mass in untreated patients; these changes occurred without effects on blood pressure or blood glucose concentration. Like AGE inhibitors and breakers, triethylenetetramine also protected rats with streptozotocin (STZ)-induced diabetes against development of cardiovascular and renal pathology, including left ventricular dysfunction and collagen accumulation, renal fibrosis, and albuminuria. Triethylenetetramine also affected the renal proteome, altering the expression of numerous proteins involved in cellular metabolism, ion transport, and oxidative stress in the kidney of rats with STZ-induced diabetes. In later studies, Baynes and Murray (46) demonstrated that triethylenetetramine inhibited structural and functional changes in the heart and kidney of Zucker diabetic rats, a model of type 2 diabetes. Consistent with the studies in type 1 animal models and clinical studies (46), protection of cardiac function in type 2 diabetic rats was achieved without effect on blood pressure or blood glucose concentration. The effect of triethylenetetramine on tissue AGES is still unknown.

Baynes and Murray (46) and Nagai et al. (47) evaluated the effects of citrate, a relatively nonspecific chelator, on progression of diabetes complications. Citrate, at the same dose as triethylenetetramine (1 g/L in drinking water), provided comparable protection against cardiac structural and functional changes in the Zucker type 2 diabetic rat. In rats with STZ-induced diabetes, citrate also inhibited albuminuria, cataractogenesis, and ketosis (47). The effects of citrate on ketosis have previously been reported, but the study of Nagai et al. (47) demonstrated that citrate also inhibited formation of two AGES, both CML and N°-(carboxyethyl)lysine (CEL), in the lens. This is the first evidence that even a common dietary chelator found in citrus fruits and drinks may inhibit AGE formation and protect against development of diabetes complications. Although citrate is known to promote intestinal absorption of copper and iron, there is no information on its overall effects on metal ion homeostasis in vivo or on AGE formation in other tissues. There are numerous reports in the literature on the benefits of plant extracts and nutraceuticals (e.g., rutin, polyphenols, quercetin, resveratrol) against diabetes complications. Some of these compounds also inhibit AGE formation in diabetic animals, and it is possible that they may work, in part, by limiting the uptake or promoting the excretion of metal ions through chelating activity.

One of the puzzling aspects of the studies is that triethylenetetramine, which is a weak iron chelator and does not appear to affect overall iron balance (45), has such an impact on diabetes complications, when both iron and copper excess are apparent in diabetes (43,48). It is also clear that Wilson disease, which is characterized by severe copper overload in tissues and high levels of chelatable copper, does not lead to the characteristic complications of diabetes, although this might be obscured by the severe hepatic disease. Although iron is strongly implicated in the pathogenesis of diabetes and its complications and although specific iron chelators, such as desferoxamine, have shown beneficial effects in treatment of diabetes both in animal models and in clinical studies (48,49), particularly in patients with iron overload diseases, the iron-chelating activity of various drugs has not been well studied, possibly because of the facile oxidation and precipitation of iron salts at neutral pH under air. It is likely that the copper and iron work together in diabetes; the disorder in copper homeostasis may lead to oxidation of iron and deposition of insoluble, but potentially redox active, ferric iron in tissues. These metals could cocatalyze oxidative stress and resultant inflammation, probably exacerbated by mitochondrial dysfunction and hypoxia in diabetes. The rapid elimination of decompartmentalized copper by triethylenetetramine would intercept the ROS-generating cycle, possibly leading to a more gradual leaching of iron from tissues and then to restoration of vascular tone.

CONCLUSIONS AND PERSPECTIVE

We have presented evidence here that the activity of AGE inhibitors and breakers on the formation of AGES and development of diabetes complications may be explained in large part by their chelating activity or the chelating activity of products of their hydrolysis and/or metabolism. In addition, we propose that the AGE-inhibitory activity of other drugs commonly used for treatment of diabetes complications, including ACEIs, ARBs and ARIs, may also be attributed to their chelation activity. These compounds, as well as AGE inhibitors and breakers, have a diverse range of structures, and other functions clearly come into play with specific compounds, e.g., antihypertensive agents; however, chelation stands out as a likely, common mechanism of their action on AGE formation. Higher doses are required for weaker and more polar chelators, such as aminoguanidine and pyridoxamine, while more hydrophobic compounds, such as the LR compounds, ACEIs, ARBs, and ARls, work effectively at lower doses, probably because of differences in bioavailability, plasma, or tissue half-lives or metabolism. Hydrophobic chelators may also penetrate and concentrate in membranous compartments and remove loosely bound metal ions from subcellular compartments. It is also possible that some chelators may shift the redox potential of iron or copper, affecting their catalytic activity in a way that could exacerbate oxidative stress and diabetes complications. To date, there are few reports on the effects of any of these compounds on metal ion homeostasis, and AGES have not been measured in renal or vascular tissues or in collagen after treatment with chelators. Such experiments are essential to test the hypothesis presented in this article that chelation is a fundamental mechanism of action of the wide range of compounds that affect AGE formation in diabetes. While we have focused this discussion on AGES and AGE inhibitors and breakers, lipoxidation reactions proceed in concert with glycoxidation and oxidation in tissues and AGE inhibitors also inhibit lipoxidation reactions (11), so it will be important to determine the extent to which the formation of ALEs, as well as other biomarkers of oxidative stress in diabetes, is inhibited by chelators. Finally, the interplay between iron and copper in oxidative stress, diabetes, and its complications deserves greater attention. Both iron and copper chelators inhibit AGE formation in model systems in vitro, and it is equally likely that these metals have both unique and complementary roles in vivo.

In conclusion, although “Quackwatch” appears among the first three URLs in a Bing, Google, or Yahoo search for “chelation therapy,” we argue that there is good reason to invest in further research on chronic, low-dose, oral chelation therapy for treatment of diabetes and its complications. This recommendation also applies to cardiovascular and neurodegenerative diseases (50) and other diseases with a chronic inflammatory component, marked by the accumulation of iron and copper, AGES and ALEs, and
protein and DNA oxidation products at sites of pathology. Chelation should inhibit the metal-catalyzed oxidation reactions and inflammatory processes leading to and resulting from accumulation of oxidative damage in tissues.

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REFERENCES

1. Goh SY, Cooper ME. Clinical review: the role of advanced glycation end products in progression and complications of diabetes. J Clin Endocrinol Metab 2008;93:1143–1152
2. Tessier FJ. The Maillard reaction in the human body. The main discoveries and factors that affect glycation. Pathol Biol (Paris) 2010;58:214–219
3. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991;40:405–412
4. Reddy VP, Beyaz A. Inhibitors of the Maillard reaction and AGE breakers as therapeutics for multiple diseases. Drug Discov Today 2006;11:646–654
5. Peyroux J, Sternberg M. Advanced glycation endproducts (AGEs): pharmacological inhibition in diabetes. Pathol Biol (Paris) 2006;54:405–419
6. Brownlee M. Vlassara H, Kooney A, Ulrich P, Cermak A. Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. Science 1986;232:1629–1632
7. Thomalley PJ. Use of aminoguanidine (Pyomageline) to prevent the formation of advanced glycation endproducts. Arch Biochem Biophys 2003a;419:1645–1653
8. Reddy VP, Beyaz A. Inhibitors of the Maillard reaction and AGE breakers as therapeutics for multiple diseases. Drug Discov Today 2006;11:646–654
9. Peyroux J, Sternberg M. Advanced glycation endproducts (AGEs): pharmacological inhibition in diabetes. Pathol Biol (Paris) 2006;54:405–419
10. Brownlee M. Vlassara H, Kooney A, Ulrich P, Cermak A. Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. Science 1986;232:1629–1632
11. Thomalley PJ. Use of aminoguanidine (Pyomageline) to prevent the formation of advanced glycation endproducts. Arch Biochem Biophys 2003a;419:1645–1653
12. Schalkwijk CG, Miyata T. Early- and advanced non-enzymatic glycation in diabetic vascular complications: the search for therapeutics. Amino Acids. 20 October 2010 [Epub ahead of print]
13. Buettner GR. Use of ascorbate as test for catalytic metals in simple buffers. Methods Enzymol 1990;186:155–127
14. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 2005;54:1615–1625
15. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res 2010;107:1058–1070
16. Balakumar P, Rohilla A, Krishan P, Solaraj P, Thangarthirupathi A. The multifaceted therapeutic potential of benfotiamine. Pharmacol Res 2010;61:482–488
17. Rahbar S, Figarola JL. Inhibitors and breakers of advanced glycation endproducts (AGEs): a review. Curr Med Chem 2002;2:135–161
18. Rahbar S, Figarola JL. Novel inhibitors of advanced glycation endproducts. Arch Biochem Biophys 2003;419:83–79
19. Figarola JL, Scott S, Loera S, et al. Prevention of early renal disease, dyslipidaemia and lipid peroxidation in STZ-diabetic rats by LR-9 and LR-74, novel AGE inhibitors. Diabetes Metab Res Rev 2005;21:533–534
20. Figarola JL, Loera S, Weng Y, Shannumug N, Natarajan R, Rahbar S. LR-90 prevents dyslipidemia and diabetic nephropathy in the Zucker diabetic fatty rat. Diabetologia 2008;51:882–891
21. Ishihara Y, Nangaku M, Takizawa S, et al. A novel class of advanced glycation inhibitors ameliorates renal and cardiovascular damage in experimental rat models. Nephrol Dial Transplant 2008;23:497–509
22. Figarola JL, Shannumug N, Natarajan R, Rahbar S. Anti-inflammatory effects of the advanced glycation end product inhibitor LR-90 in human monocytes. Diabetes 2007;56:647–655
23. Price DL, Rhett PM, Thorpe SR, Baynes JW. Chelating activity of advanced glycation end-product inhibitors. J Biol Chem 2001;276:48907–48972
24. Miyata T, van Ypersele de Strihou C, Ueda Y, et al. Angiotensin II receptor antagonists and angiotensin-converting enzyme inhibitors lower in vitro the formation of advanced glycation end products: biochemical mechanisms. J Am Soc Nephrol 2002;13:2478–2487
25. Miyata T, van Ypersele de Strihou C. Angiotensin II receptor blockers and angiotensin converting enzyme inhibitors: implication of radical scavenging and transition metal chelation in inhibition of advanced glycation end product formation. Arch Biochem Biophys 2005;448:56–54
26. Forbes JM, Cooper ME, Thallas V, et al. Reduction of the accumulation of advanced glycation end products by ACE inhibition in experimental diabetic nephropathy. Diabetes 2002;51:3274–3282
27. Liu XP, Pang YJ, Zhu WW, et al. Benazepril, an angiotensin-converting enzyme inhibitor, alleviates renal injury in spontaneously hypertensive rats by inhibiting advanced glycation end-product-mediated pathways. Clin Exp Pharmacol Physiol 2009;36:287–296
28. Monacelli F, Poggi A, Storace D, et al. Effects of valsartan therapy on protein glycoxidation. Metab 2005;54:1619–1625
29. Wihler C, Schäfer S, Schmid K, et al. Renal accumulation and clearance of advanced glycation end-products in type 2 diabetic nephropathy: effect of angiotensin-converting enzyme and vasoprotective inhibition. Diabetologia 2005;48:1645–1653
30. Ishihara Y, Sada T, Yanagisawa H, et al. A novel Sartan derivative with very low angiotensin II type 1 receptor affinity protects the kidney in type 2 diabetic rats. Arterioscler Thromb Vasc Biol 2008;28:1767–1773
31. Nangaku M, Miyata T, Sada T, et al. Anti-hypertensive agents inhibit in vivo the formation of advanced glycation end products and improve renal damage in a type 2 diabetic nephropathy rat model. J Am Soc Nephrol 2003;14:1212–1222
32. Miyata T, van Ypersele de Strihou C. Renoprotection of angiotensin receptor blockers: beyond blood pressure lowering. Nephrol Dial Transplant 2006;21:846–849
33. Vasam S, Zhang X, Zhang X, et al. An agent cleaving glucose-derived protein crosslinks in vivo and in vitro. Nature 1996;382:275–278
34. Wolfenbuttel BH, Boulanger CM, Crijns FR, et al. Breakers of advanced glycation end products restore large artery properties in experimental diabetes. Proc Natl Acad Sci USA 1998;95:4630–4634
35. Joshi D, Gupta R, Dubey A, et al. TRC4186, a novel AGE-breaker, improves diabetic cardiomyopathy and nephropathy in Ob-ZSF1 model of type 2 diabetes. J Cardiovasc Pharmacol 2009;54:72–81
36. Chandra KP, Shikhalwalla AK, Kotecha J, et al. Phase I clinical studies of the advanced glycation end product (AGE)-breaker TRC4186: safety, tolerability and pharmacokinetics in healthy subjects. Clin Drug Investig 2009;29:559–575
37. Susid C. Cross-link breakers as a new therapeutic approach to cardiovascular disease. Biochem Soc Trans 2007;35:853–856
38. Yang S, Lithchfield JE, Baynes JW. AGE-breakers cleave model compounds, but do not break Maillard crosslinks in skin and tail collagen from diabetic rats. Arch Biochem Biophys 2005;432:42–46
39. El-Kabbani O, Ruiz F, Darmanin C, Chung RP. Aldose reductase structures: implications for mechanism and inhibition. Cell Mol Life Sci 2004;61:750–762
40. Ou P, Nourooz-Zadeh J, Tritschler HJ, Wolff S. Activation of aldose reductase in rat lens and metal-ion chelation by aldose reductase inhibitors and lipoic acid. Free Radic Res 1996;25:337–346
41. Nakamura J, Hamada Y, Chaya S, et al. Transition metals and polyol pathway in the development of diabetic neuropathy in rats. Diabetes Metab Res Rev 2002;18:395–402
42. Hallam KM, Li Q, Ananthakrishnan R, et al. Aldose reductase and AGE-RAGE pathways: central roles in the pathogenesis of vascular dysfunction in aging rats. Aging Cell 2010;9:776–784
43. Zheng Y, Li XK, Wang Y, Cai L. The role of zinc, copper and iron in the pathogenesis of diabetes and diabetic complications: therapeutic effects by chelators. Hemoglobin 2008;32:135–145
44. Urui-Adams JY, Keen CL. Copper, oxidative stress, and human health. Mol Asp Med 2005;26:268–298
45. Cooper GJ. Therapeutic potential of copper chelation with triethylene-tetramine in managing diabetes mellitus and Alzheimer’s disease. Drugs 2011;71:1281–1320
46. Baynes JW, Murray DB. The metal chelators, trientine and citrate, inhibit the development of cardiac pathology in the Zucker diabetic rat. Exp Diab Res 2009;2009:696378
47. Nagai R, Nagai M, Shimasaki S, Baynes JW, Fujiwara Y. Citric acid inhibits development of cataracts, proteinuria and ketosis in streptozotocin (type 1) diabetic rats. Biochem Biophys Res Commun 2010;393:118–122
48. Thangarajah H, Yao D, Chang E, et al. The molecular basis for impaired hypoxia-induced VEGF expression in diabetic tissues. Proc Natl Acad Sci USA 2009;106:13505–13510
49. Liu Q, Sun L, Tan Y, Wang G, Lin X, Cai L. Role of iron deficiency and overload in the pathogenesis of diabetes and diabetic complications. Curr Med Chem 2009;16:113–129
50. Jomova K, Vondrakova D, Lawson M, Valko M. Metals, oxidative stress and neurodegenerative disorders. Mol Cell Biochem 2010;345:91–104