

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. *Diabetes* 61:549–559, 2012

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 “autooxidative glycosylation” and “glycooxidation” (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).

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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 dicarbonyl 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.

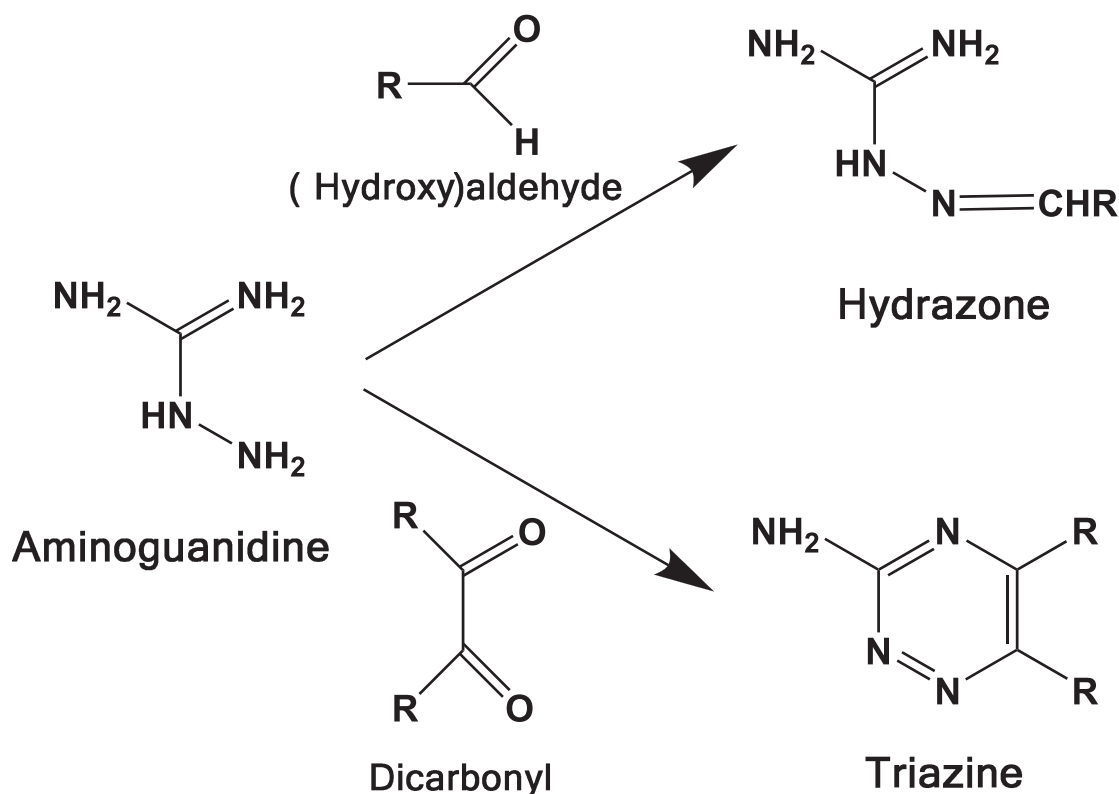


FIG. 1. Proposed mechanisms of action of aminoguanidine as a carbonyl and dicarbonyl trap. At the top, aminoguanidine reacts with carbonyl or α -hydroxycarbonyl sugars or intermediates to form a hydrazone. At the bottom, aminoguanidine reacts with a dicarbonyl compound to form a triazine.

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) (Table 1), 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 autoxidized 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 $\leq 5\%$ 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 $\mu\text{mol/L}$ (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 autoxidative glycosylation and glycooxidation 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 mmol/L range, while carnosine, diaminophenazine, OPB-9195, and tenilsetam are effective in the 5–50 $\mu\text{mol/L}$ 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

AGE 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 $\mu\text{mol/L}$ range (Table 1). Forbes et al. (26) demonstrated that the ACEI ramipril, at $\sim 10 \mu\text{mol/L}$ in drinking water ($\sim 0.1\%$ 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 (nondiabetic) 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 $\mu\text{mol/L}$ for the ACEI temocaprilat (24) and $< 10 \mu\text{mol/L}$ for AVE8048, the active metabolite of the combination ACEI/vasopeptidase inhibitor AVE7688, which was a more potent AGE inhibitor (29). Izuhara 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 ($\sim 3 \mu\text{mol/L}$) 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). Nangaku 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 $\sim 1 \mu\text{mol/L}$ (31). In their comparison of the ARB olmesartan ($IC_{50} \sim 5 \mu\text{mol/L}$) with hydralazine, the authors noted peak plasma concentration of $\sim 10 \mu\text{mol/L}$ for both drugs in clinical studies, indicating that tissue concentrations of the drugs should be sufficient to inhibit metal-catalyzed oxidation and glycooxidation 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

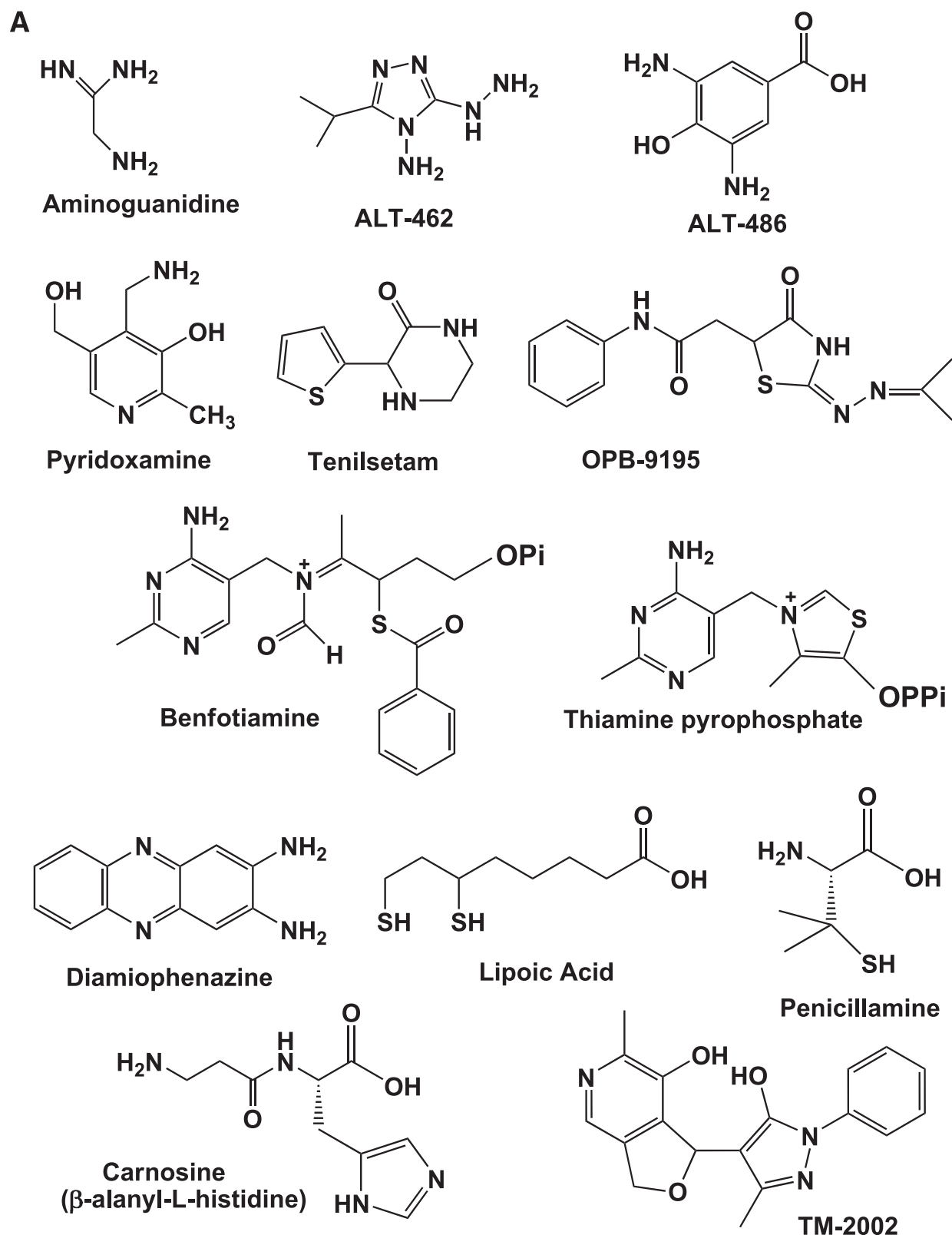


FIG. 2. Structures of AGE inhibitors. A: First-generation AGE inhibitors. B: Novel LR compounds (adapted from Rahbar and Figarola [18]).

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*-phenacylthiazolium bromide (PTB) (33), its dimethylthiazolium analog alagebrium (ALT)-711 (34), and recently described pyridinium analogs

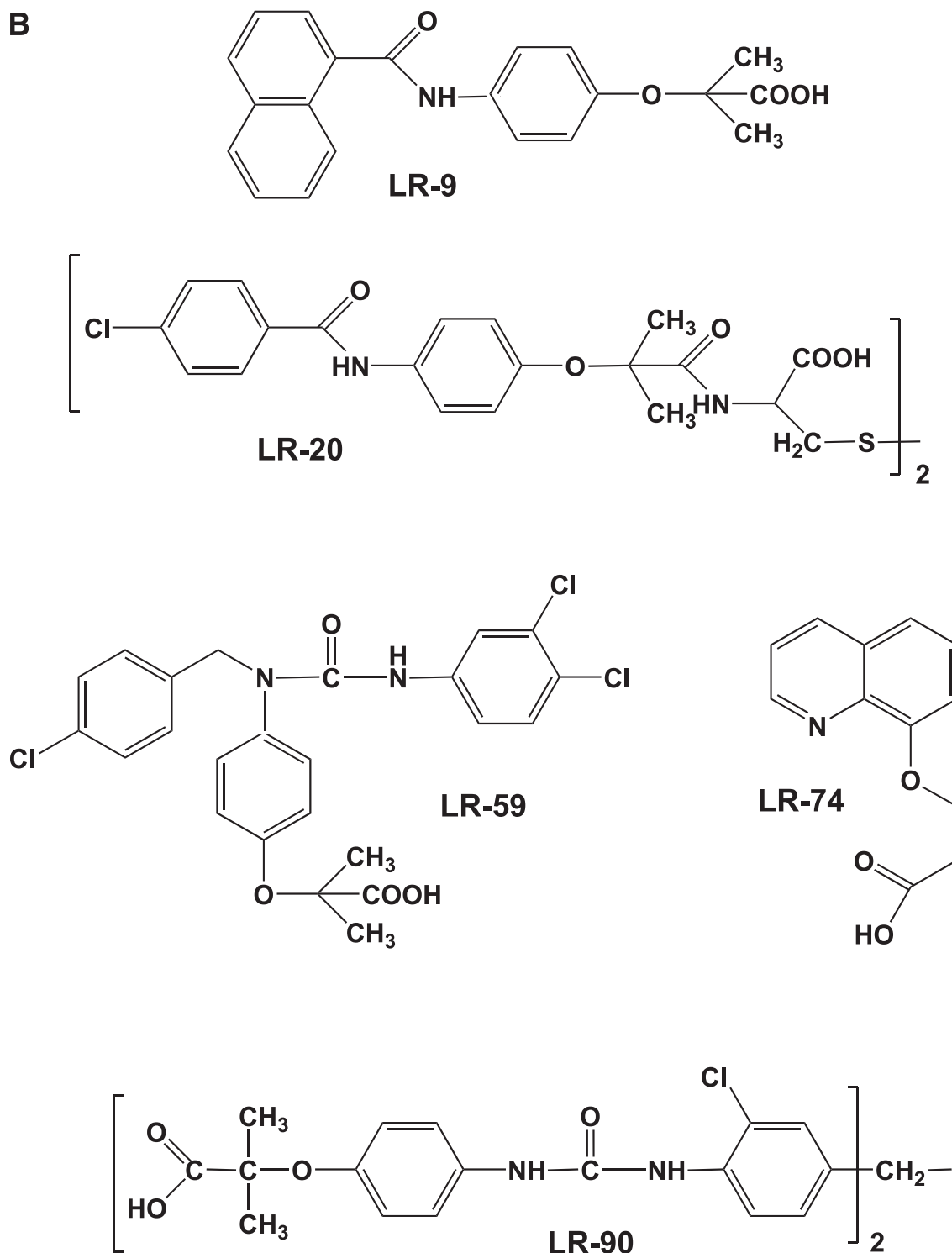


FIG. 2. Continued

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).

TABLE 1
IC₅₀ of various compounds that inhibit metal-catalyzed oxidation of ascorbate*

	IC ₅₀ (μmol/L)	Reference
AGE inhibitors		
Aminoguanidine	1,000–4,600	17, 23, 24
Pyridoxamine	1,000–2,500	17, 23, 24
Carnosine	0.5–7	17, 23
Diaminophenazine	50	23
Edaravone	114	21
Lipoic acid	125	17
OPB-9195	3–5	23, 24
TM2002	84	21
Tenilsetam	25	23
LR-90	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	—

TETA, triethylenetetramine. *Based on inhibition of metal-catalyzed oxidation of ascorbate in neutral phosphate buffer, as previously described by Buettner (ref. 13).

Yang et al. (38) demonstrated that although AGE breakers cleaved dicarbonyl structures in model compounds, they did not cleave cross-links in insoluble skin or tendon collagen of diabetic rats or cleave cross-links in RNase polymerized by reaction with glucose in vitro. In all of the studies demonstrating the activity of AGE breakers in vitro, fresh rat skin or tail collagens (unprocessed by dialysis or acid extraction to remove labile intermediates or cross-links) were used for analysis of cross-link breaking activity. In contrast, the lack of AGE-breaking activity was demonstrated with the acetic acid-extracted insoluble fraction of skin collagen, which would lack the labile (reversible) intermediates and cross-links. Similarly, red cells may have proteins bound to their surface membranes by labile Schiff base (imine) bonds. These, like the labile Schiff base fraction of glycated hemoglobin, would dissociate during incubation with AGE breakers. Overall, it is unlikely that AGE breakers react according to the reaction scheme shown in Fig. 3. Indeed, one of the proposed products of AGE breaking is the AGE N^ε-(carboxymethyl)lysine (CML), which has been identified as a ligand for RAGE and would thereby initiate a proinflammatory response. In addition, the protein is left with a four-carbon carbohydrate fragment with a reactive aldehyde group, which would participate in further AGE formation. Thus, it is difficult to rationalize beneficial effects from the proposed mechanism of action of AGE breakers.

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 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 convincingly 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 glycoxidation 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 (39). 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 hydroxyl 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 (43–45). Cooper (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 ~5% reversion of left ventricular mass toward normal in type 2 diabetic patients compared with an increase of 3% in left

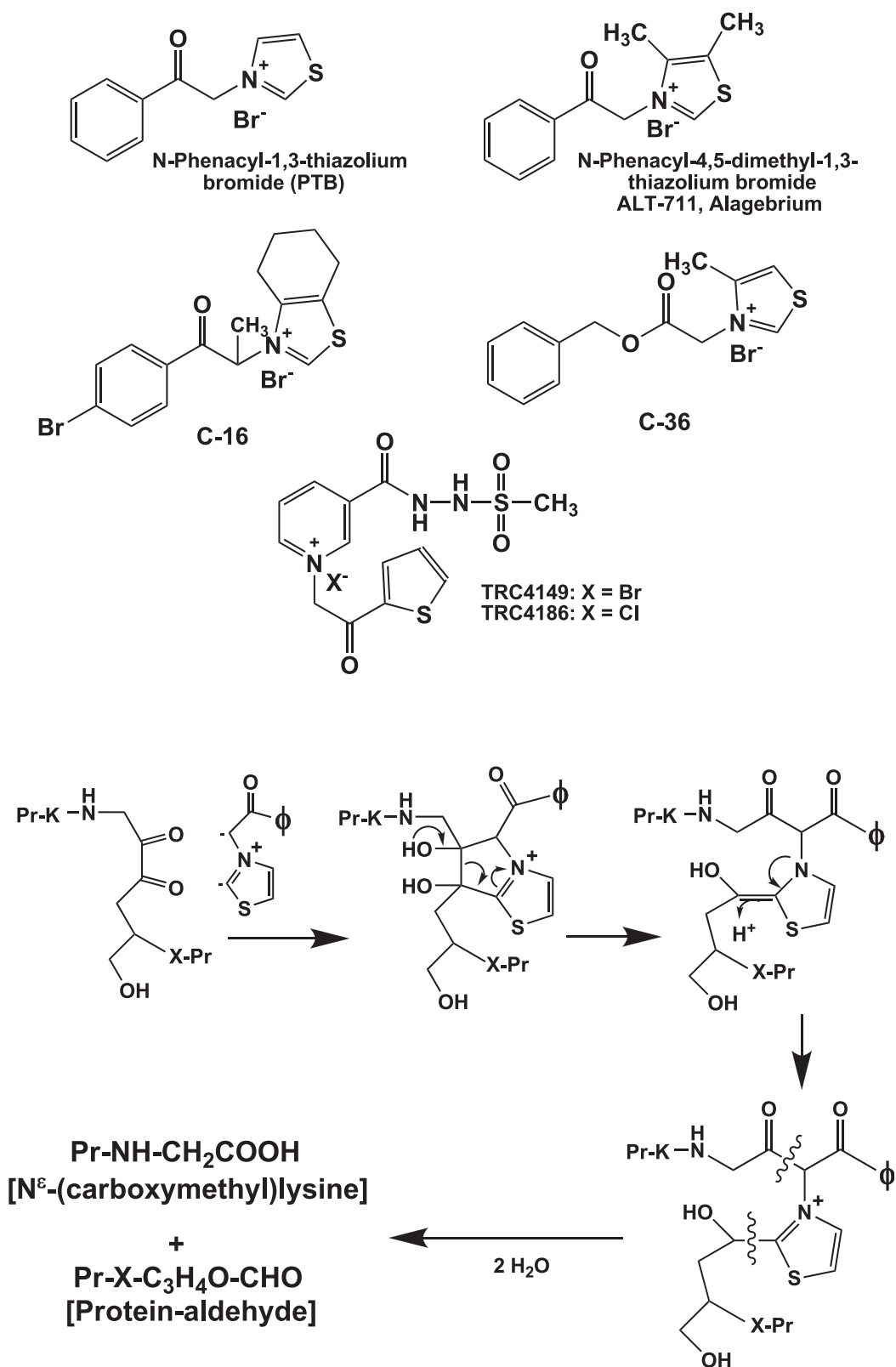


FIG. 3. Structures (*top panel*) and proposed mechanism of action (*bottom panel*) of AGE breakers. According to this scheme, the dinucleophilic AGE breaker adds across a dicarbonyl AGE cross-link, followed by an internal rearrangement to cleave the dicarbonyl bond, breaking the cross-link. Following 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].)

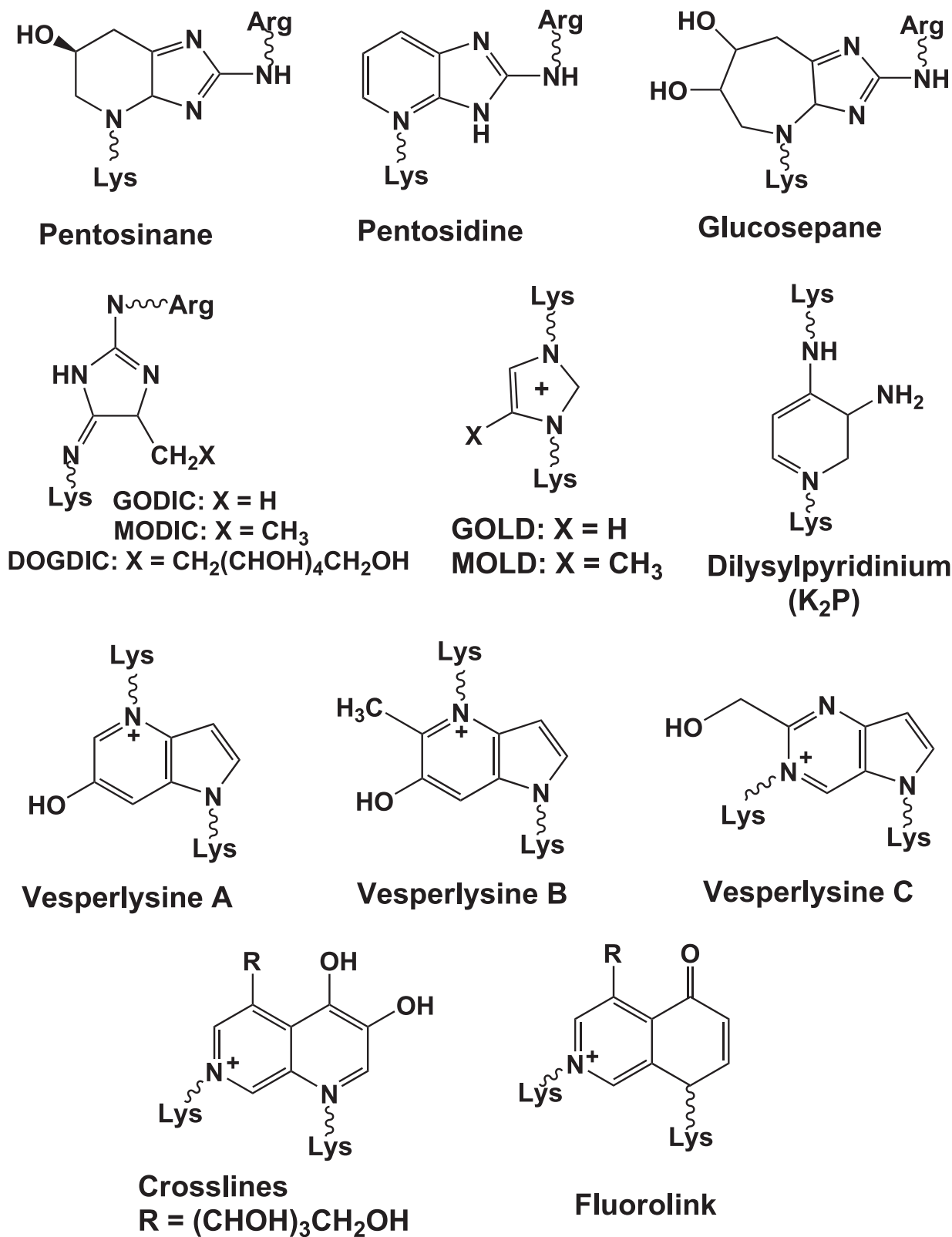


FIG. 4. Structures of AGE cross-links identified in tissue proteins. Lys-Arg cross-links are shown in the *top panel* and Lys-Lys cross-links in the *lower panel*. Compounds with two or three carbon cross-links, e.g., GODIC, MODIC, GOLD, MOLD, and K₂P, may be derived from both carbohydrates and lipids, i.e., they are AGE/ALEs. All of these compounds are considered to be irreversible AGE cross-links in proteins. Pentosidine, 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^ε-(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 ARIs, 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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