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Inhibitors of the Maillard reaction and AGE breakers as therapeutics for multiple diseases

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The Maillard reaction is a complex series of reactions that involve reducing-sugars and proteins, giving a multitude of end-products that are known as advanced glycation end-products (AGEs). AGEs can contribute to the pathogenesis of diabetes and neurological diseases such as Alzheimer's disease. AGEs also play a major role in vascular stiffening, atherosclerosis, osteoarthritis, inflammatory arthritis and cataracts. Thus, AGE inhibitors and AGE breakers offer a potential strategy as therapeutics for diverse diseases. Various AGE inhibitors have been developed in recent years, and their underlying mechanism is based on the attenuation of glycoxidation and/or oxidative stress by the sequestration of metal ions, reactive 1,2-dicarbonyl compounds, and reactive oxygen and reactive nitrogen species.

The Maillard reaction, also referred to as nonenzymatic glycation or browning, was characterized in 1912 by a French scientist, Louis Camille Maillard. In the pioneering work of Maillard, yellow–brown colors were observed when reducing-sugars were heated with amino acids [1]. The Maillard reaction is widely used in the food industry to control food texture; however, it is now acknowledged to be involved in the pathogenesis of various diseases, particularly diabetes mellitus and neurodegenerative diseases such as Alzheimer's disease [2–7]. The Maillard reaction is not actually a single reaction, but a series of nonenzymatic reactions involving the reaction between carbonyl groups of reducing-sugars with amino groups of proteins, enzymes, nucleic acids or phospholipids, forming Schiff bases and followed by their Amadori rearrangement and subsequent oxidative modifications (glycoxidations) that are induced by reactive oxygen species (ROS) and reactive nitrogen species (RNS). The end result of these complex series of reactions is the formation of AGEs. Glycoxidation of the Amadori products, formed in the initial phase of the Maillard reaction, results in the formation of reactive 1,2-dicarbonyl compounds such as glyoxal and glucosone. Autoxidations of glucose, or the corresponding Schiff base and the Amadori products, also give rise to other 1,2-dicarbonyl compounds such as methylglyoxal, 1-deoxyglucosone (1-dG) and 3-deoxyglucosone (3-dG). The increased electrophilicity of these 1,2-dicarbonyl compounds results in their relatively fast reactions with amino groups of proteins, and subsequent glycoxidative modifications result in the formation of the crosslinked proteins (Figure 1). Some of the AGEs are intensely colored compounds and have typical fluorescence characteristics (excitation at 330 nm and emission at 400 nm). However, not all of the AGEs are derived from protein crosslinks. N^{ε}-carboxymethyllysine (CML), for example, is derived from the modification of a lysine residue of a single protein. Such protein modifications can cause enzyme inactivation. A variety of AGEs, formed *in vitro* and *in vivo*, have been isolated and structurally characterized [4].

Formation of AGEs is a relatively slow process under physiological conditions, and, therefore, accumulation of AGEs is prominent in long-lived proteins such as lens crystallins and tissue collagens. As a consequence of the formation of AGEs, biologically active proteins and enzymes are deactivated through inter- and intra-molecular crosslinks. Further, AGEs complex with metal ions (such as Cu^{+} and Fe^{2+}) and can provide catalytic sites for the formation of ROS and RNS [8]. Diabetes is a major source of AGEs *in vivo*. In addition to their natural formation, causes of the formation of AGEs in the human body are smoking and a diet enriched with AGEs or advanced lipoxidation end-products (ALEs) [9,10]. There is a significant correlation between ingested AGEs and circulating AGEs in humans [11]. Incidences of lung and cardiovascular disease, as well as cataracts, are relatively higher in smokers than in non-smokers, which might be linked to the AGEs that are associated with smoking [10]. Plasma from smokers

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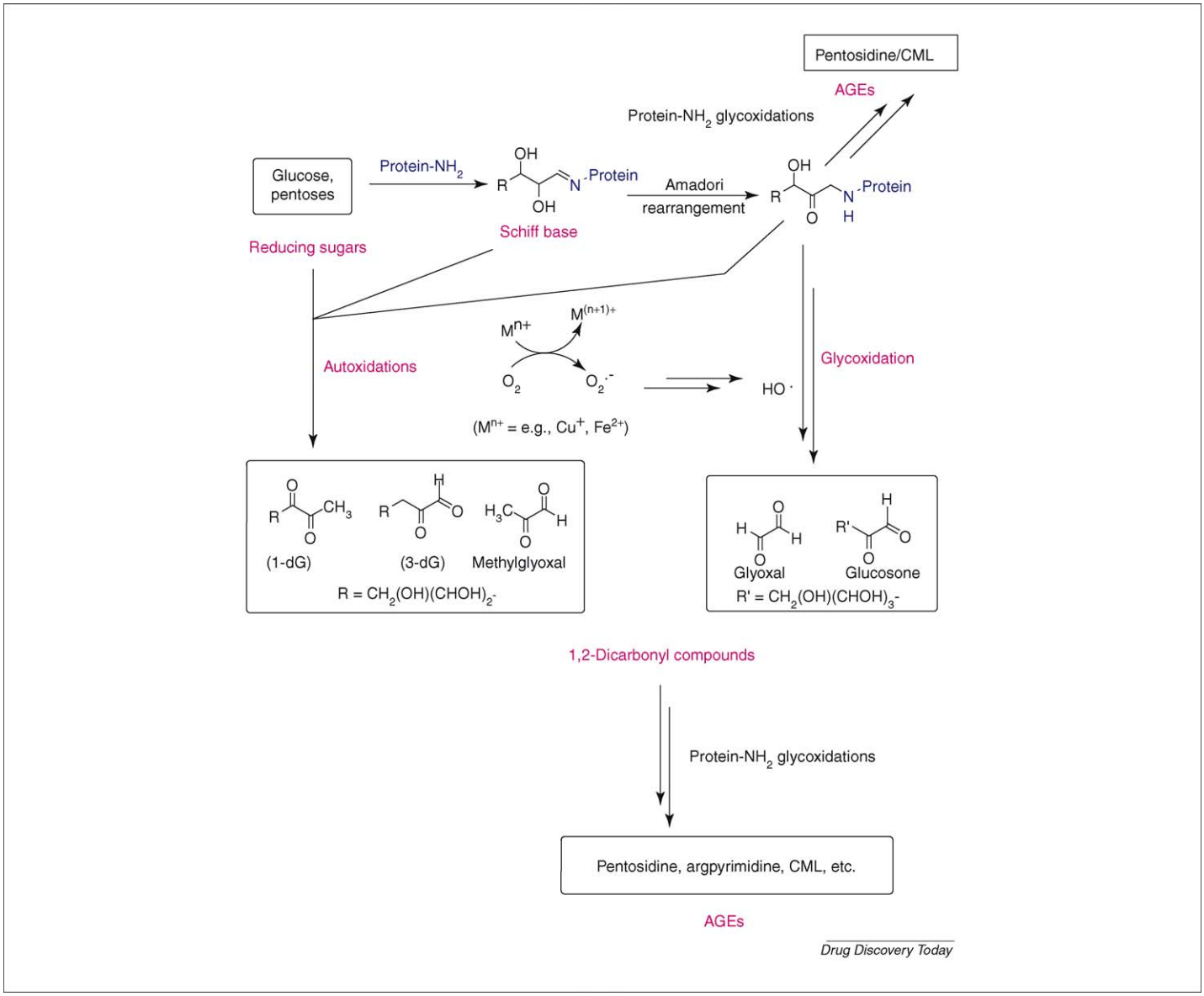

FIGURE 1

Schematic representation of the formation of advanced glycation end products (AGEs) through the Maillard reaction. Nonenzymatic reactions of the carbonyl groups of reducing sugars with primary amino groups of the proteins produce their corresponding Schiff bases, which undergo Amadori rearragements to give protein-derived aminomethyl ketones. Transition-metal-ion-catalyzed glycoxidations of the Amadori compounds, involving reactive oxygen species (ROS) and reactive nitrogen species (RNS), give 1,2-dicarbonyl compounds such as glyoxal. Other sources of 1,2-dicarbonyl compounds include autoxidations of glucose, Schiff bases or the corresponding Amadori products. These highly reactive dicarbonyl compounds further react with surrounding protein amino groups, and undergo further glycoxidations forming a variety of protein crosslinks or protein modifications, and are referred to as AGEs. Pentosidine and N^{ε}-carboxymethyllysine (CML) can also be derived directly from the Amadori products. Typically, the lysine and arginine residues of proteins are involved in the formation of the AGEs, which, because of their involvement in enzyme active sites, can result in enzyme deactivation.

contains relatively high concentrations of nornicotine-modified proteins [12]. Studies with human gingival cells grown in tissue culture indicate that nornicotine (a constituent of tobacco and a metabolite of nicotine) upregulates the expression of the AGE receptor (RAGE). Therefore, RAGEs exacerbated by smoking could play a significant role in periodontal disease [13].

The levels of AGEs (e.g. pentosidine) correlate with several markers of inflammation in rheumatoid arthritis (RA) [e.g. serum levels of interleukin-6 (IL-6) and C-reactive protein] [14,15]. Elevated levels of AGEs are particularly prominent in diabetes. By binding to RAGEs, AGE-modified proteins induce changes that promote matrix overproduction, focal thrombosis and vasoconstriction [16]. Thus, pharmaceutical agents that inhibit the formation of AGEs or act as AGE breakers (i.e. AGE-reversing agents) might provide attractive therapeutics for various, seemingly unrelated, diseases [7,17].

Formation of AGEs *in vivo*

Reducing-sugars, such as glucose and ribose, usually undergo Maillard reactions with lysine and arginine residues of proteins, forming protein crosslinks. One of the arginine–lysine-derived AGEs, referred to as pentosidine, was first isolated from brain tissues by Monnier and Sell, and has been useful as a biomarker of age-related diseases [7,18]. Pentosidine can be synthesized

in vitro by incubating a mixture of arginine, lysine and reducing-sugars such as glucose, ribose and ascorbate. It has recently been shown that pentosidine can be formed by incubating short-chain carbohydrates (such as glyoxal, methylglhyoxal and glycoaldehyde) with a mixture of N_{α}-acetylarginine and N_{α}-acetyllysine [19]. Thus, pentosidine is derived either directly from the Amadori intermediate (through reaction with arginine residues) or from the 1,2-dicarbonyl compounds (e.g. glyoxal) that are formed through glycoxidation of the Amadori intermediate. Similarly, CML can be derived through both of these pathways. Methylglyoxal rapidly reacts with arginine-containing proteins to give a protein modification, argpyrimidine [20]. Pentosidine and argpyrimidine concentrations are elevated in the serum of diabetic patients and in brunescent cataractous lenses, and serve as markers of the progression of disease [20]. Pentosidine levels have also been correlated with arterial stiffness [21]. 3-dG reacts with the lysine residues of proteins to give pyrraline, which is associated with cataractous human lens α-crystallins [22]. Using antibodies specific to pentosidine and pyrraline, Smith and co-workers [23] were able to detect these AGEs in the Rosenthal fibers in cases of Alexander's disease, a genetic degenerative disorder of the central nervous system. The protein αB-crystallin is the major component of Rosenthal fibers. Data suggest that αB-crytallins of Rosenthal fibers are targets for AGE modifications, as has also been observed for diabetic and aged-lens crystallins. Therefore, AGE modifications in Rosenthal fibers (e.g. pentosidine and pyrraline modifications) could be crucial to the pathogenesis of Alexander's disease [23].

Glyoxal and methylglyoxal also form crosslinks between two lysine residues, known as glyoxal–lysine dimer (GOLD) and methylglyoxal–lysine dimer (MOLD), respectively. Cataractous lenses showed significantly higher levels of GOLD and MOLD compared with normal lenses from age-matched subjects [24]. They were also elevated in diabetic plasma proteins. CML is one of the stable AGEs, and has been isolated from the human cataractous lenses and tissues [25]. CML is derived from the glyoxal modification of lysine residues or through the oxidative modification of the Amadori intermediate. It is also found in the brains of Alzheimer's disease patients [26]. Arginine residues can be modified by the reaction of the guanidino group with methylglyoxal to give hydroimidazolone [N_{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)ornithine] [27]. These glycoxidative modifications of lysine and arginine residues dramatically alter enzyme activities because they are frequently involved in enzyme active sites. Glyoxal- and methylglyoxal-derived lysine–arginine crosslinks (GODIC and MODIC, respectively) are additional markers of pathophysiological processes [28]. For example, GODIC has been shown to be elevated significantly in brunescent lenses [28]. Recently, a lysine–arginine crosslink, glucosepane, has been isolated from the Maillard reactions of glucose with arginine and lysine residues [29,30]. Glucosepane was shown to be a major protein crosslink of the senescent human extracellular matrix, and is elevated in diabetes [31]. Structures of representative AGEs are shown in Figure 2.

AGEs in aging and disease

The formation of AGEs progressively increases with normal aging, even in the absence of disease. However, they are formed at accelerated rates in diabetes. AGEs are not only markers but also important causative factors for the pathogenesis of diabetes [3], cataracts [32], atherosclerosis [33], diabetic nephropathy [34] and neurodegenerative diseases, including Alzheimer's disease [35], Parkinson's disease [36] and amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease) [37,38]. Coloration of the human lens in certain types of cataracts is, among other factors, related to the formation of AGEs on lens proteins. The UVA-absorbing AGEs, OP-lysine [2-ammonio-6-(3-oxidopyridinium-1-yl)hexanoate] and argpyrimidine, are abundant in lenses (Figure 2) [39].

Immunohistochemical studies in cases of neurodegenerative diseases show localization of AGEs in amyloid plaques and neurofibrillary tangles (NFTs) [37,38,40,41]. Associations of AGEs with Pick bodies in Pick's disease, and granulovacuolar degeneration in a variety of neurodegenerative diseases, have also been demonstrated, thus implying that AGEs might contribute to neuronal dysfunction and the progression of neurodegenerative diseases including Alzheimer's disease [38].

The levels of methylglyoxal and glyoxal, the precursors of AGEs, are elevated in the cerebrospinal fluids of Alzheimer's disease patients, in comparison with healthy controls. Concomitantly, the levels of glyoxalase I and glyoxalase II enzymes, the glutathione-dependent enzyme systems responsible for detoxification of glyoxal and methylglyoxal, are elevated in the cerebral spinal fluid from Alzheimer's disease patients [42].

Although glycation and AGEs are implicated in the progression of various diseases it is most prominent in diabetes and diabetes-associated vascular disorders [3]. Plasma or serum AGE levels are relatively high in patients with hyperglycemia and/or oxidative stress, especially in diabetes, RA, systemic lupus erythematosus (SLE) and renal insufficiency [9,43–45]. This is a problem that will continue to rise because it is estimated that 300 million people world-wide will have diabetes by 2025 [2].

Accumulation of AGEs has enormous impact in the cardiovascular system and can contribute to structural and physiological changes, including arterial and myocardial stiffness, endothelial dysfunction, remodeled vascular injury responses and atherosclerotic plaque formation [46]. The effects of AGEs on the cardiovascular system involve crosslinking of collagen and circulating proteins [e.g. low density lipoproteins (LDLs)], and impaired cellular nitric oxide signaling through AGE–RAGE interactions [46].

The interaction of AGEs with their receptors, RAGEs, results in enhanced oxidative stress through signal transduction mechanisms. AGE–RAGE interaction is, at least in part, responsible for the development of late diabetic complications, such as neuropathy and nephropathy, and cardiovascular complications [47]. RAGE is a member of the immunoglobulin superfamily of proteins. RAGE antagonists such as nifedipine, a dihydropyridine-based calcium antagonist, have been shown to inhibit the overexpression of RAGEs in AGE-exposed endothelial cells, presumably by suppressing the generation of ROS [48]. Soluble RAGEs (sRAGEs), the extracellular ligand-binding domains of RAGEs, compete with RAGEs in binding AGEs. Binding of sRAGEs with AGEs, in contrast to AGE–RAGE interactions, does not result in inflammatory signal transduction. Thus, sRAGEs offer protective effects as AGE inhibitors, potentially applicable for the treatment of various AGE-related diseases including diabetic cardiovascular complications and diabetic kidney disease [47,48].

Kidneys play an important role in the metabolism of AGEs. Renal proximal tubule cells absorb and catabolize AGEs from the

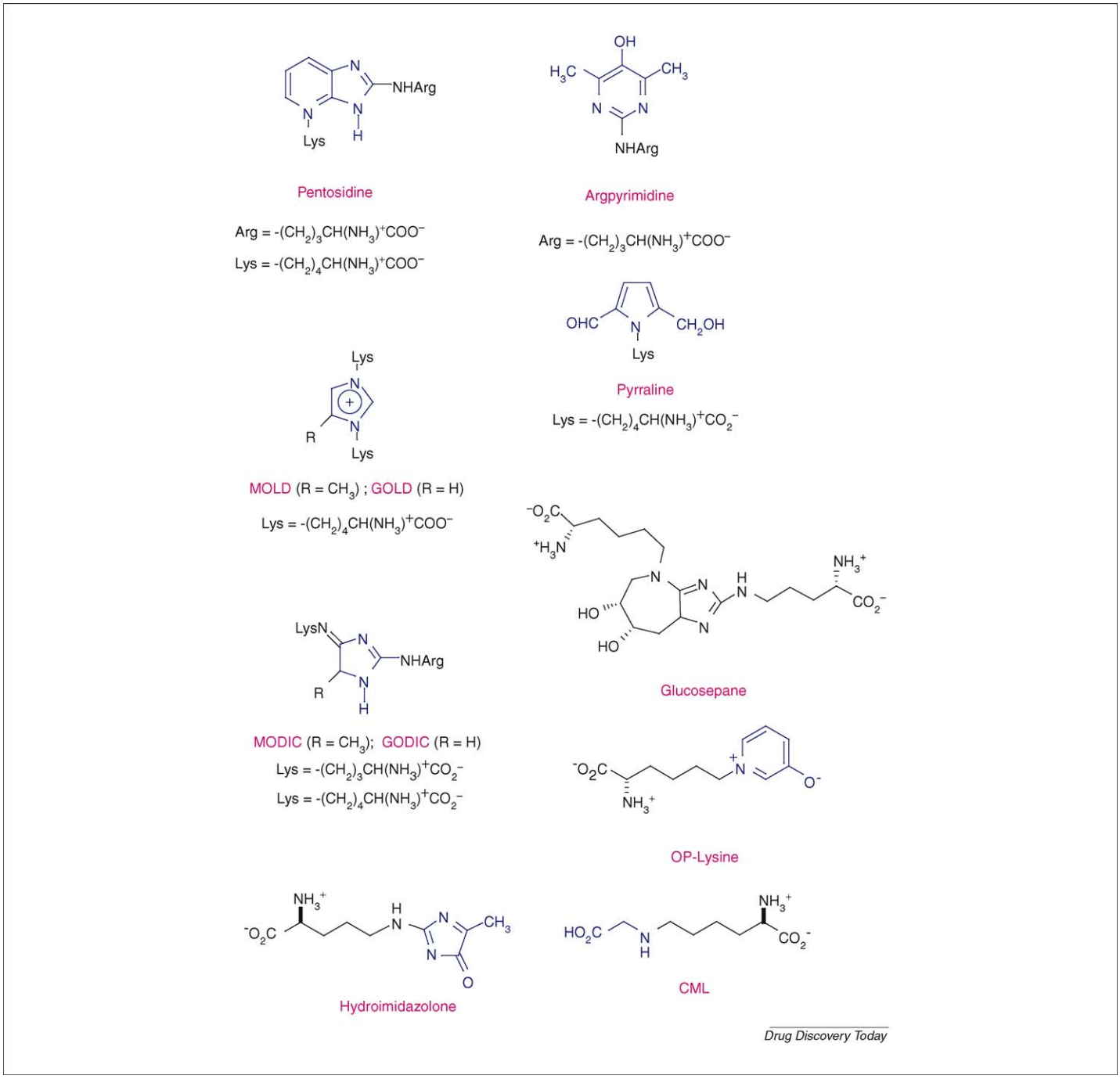

FIGURE 2

Structures of selected advanced glycation end products (AGEs). Maillard reactions involving the reactions of carbonyl groups of reducing sugars and the amino groups of proteins, followed by oxidative modifications, generate a multitude of AGEs. A limited number of AGEs have been isolated and spectroscopically characterized from *in vivo* and *in vitro* Maillard reactions. AGEs, pentosidine, glucosepane, Glyoxal-derived lysine–arginine crosslinks (GODIC), methylglyoxal-derived lysine–arginine crosslinks (MODIC), glyoxal–lysine dimer (GOLD) and methylglyoxal–lysine dimer (MOLD) represent protein crosslinks, whereas argpyrimidine, pyrraline, N^{ε}-carboxymethyllysine (CML) and OP-lysine represent single-protein modifications. AGEs are involved in a variety of age-related diseases, including Alzheimer's disease, diabetes and related vascular complications. Formation of AGEs progressively increases in the human body even with normal aging, but AGEs are formed at accelerated rates in diabetes as a result of the associated hyperglycemia and oxidative stress.

glomerular filtrate [49,50]. AGEs accumulate in the serum of patients with chronic renal failure (CRF) as a result of reduced renal metabolism of AGEs. This process leads to uremic complications including dialysis-related amyloidosis [51]. Further, activation of RAGE through binding to AGEs causes expression of vascular endothelial growth factor and enhanced activation of inflammatory cells in the diabetic glomerulus, leading to the cause of albuminuria and glomerulosclerosis [52]. Disrupting the binding of AGEs (e.g. CML) to RAGEs, for example using sRAGEs, could, therefore, prove to be of value as a therapeutic intervention in diabetic nephropathy [53].

Maillard reactions are also implicated in RA and osteoarthritis (OA). The level of AGEs is significantly elevated in both RA and OA patients [54,55]. RA is a chronic disease, which has an unknown

cause but is characterized by persistent articular and systemic inflammation, pain, stiffness, swelling and, sometimes, joint destruction. OA is one of the most common diseases of older people, but the origin of OA is not clearly known [56–58]. OA causes progressive destruction of the articular cartilage in the joint, which results in impaired joint motion, severe pain and disability [57,59]. AGE accumulation is most prone to long-lived proteins (e.g. human articular cartilage collagens, where $t_{1/2}$ is ~117 years) [60]. It has been found that pentosidine levels increase 50-fold in human cartilage collagen between 20 and 80 years of age [61].

Some of the AGE effects are mediated by RAGEs, including lactoferrin, scavenger receptors types I and II, oligosaccharyl transferase-48 (OST-48), 80K-H phosphoprotein, galectin-3 and CD36 [62–65]. RAGEs transduce the effects of AGEs by signal transduction [66], inducing formation of proinflammatory cytokines, such as interleukin-6 (IL-6), through the activation of nuclear factor κB (NF-κB). The levels of AGEs, such as pentosidine in sera and synovial fluid from RA patients, correlate with levels of IL-6 [67]. sRAGEs, by contrast, do not involve signal transduction and AGEs bind competitively with RAGEs. The AGE–RAGE-mediated inflammation can be avoided by the competitive binding of sRAGEs with AGEs. In accordance with this, there is a marked decrease in the levels of sRAGEs in the synovial fluid and sera of RA patients [67]. In mouse models of RA, treatment with sRAGEs results in significant reduction in inflammation and cartilage- and/or bone-destruction [67]. RAGE antagonists such as nifedipine could have therapeutic potentials in AGE-related diseases including RA, diabetic vascular complication and Alzheimer's disease [48,68].

AGE-dependent oxidative stress

AGEs exacerbate intracellular oxidative stress, leading to the enhanced production of ROS, which could themselves be involved in cell damage [37,69–71]. For example, AGEs induce apoptosis in cultured human umbilical vein endothelial cells [72,73]. Age-related diseases such as obesity, diabetes, cardiovascular and renal disorders, and Alzheimer's disease have common increases in oxidative stress. Amyloid β peptide (Aβ), in addition to AGEs, is also a ligand for RAGE, and RAGEs are upregulated in neuronal cells of Alzheimer's disease patients in response to the elevated levels of Aβ [74]. RAGE-mediated oxidative stress is also partially responsible for diabetic vascular complications [75–77]. Although the precise mechanism by which AGEs contribute to the atherogenesis is not clear, oxidative stress induced by collagen-linked AGEs within the vessel wall could contribute to the atherogenesis, as well as vascular perturbation in established disease [77]. Further, intracellular oxidative stress is generated by the binding of AGEs to RAGEs, through the activation of the redox-sensitive transcription factor NF-κB [44,45,78], which upregulates the expression of inflammatory cytokines and inducible nitric oxide synthase (iNOS). The extent of the activation of NF-κB correlates with the severity of the hyperglycemia [44].

Oxidative stress affects macromolecules such as oligosaccharides and polysaccharides, lipids, proteins and DNA, which, in the case of Alzheimer's disease patients, causes irreversible neuronal dysfunction [79]. It has been shown that CML is co-localized with hexitol-lysine, a reduced Amadori intermediate, implying that glycation might be an early event in Alzheimer's disease [26,79]. Oxidative stress can modify these macromolecules, leading to increased levels of 8-hydroxy-2-deoxyguanosine and 8-hydroxyguanosine (markers of DNA and RNA damage, respectively), increased levels of protein carbonyls and nitrotyrosine residues in proteins, and increased levels of thiobarbituric-acid-reactive substances such as malondialdehyde [4-hydroxy-*trans*-2-nonenal (HNE)] [80] in susceptible neurons of Alzheimer's disease patients.

AGE inhibitors

AGE inhibition through attenuation of glycoxidations and carbonyl trapping

In view of the wide occurrence of AGEs and the oxidative stress derived from them in a variety of diseases, it would be of great interest to identify and develop AGE inhibitors that can suppress the formation of AGEs, either through preventing glycoxidations or by sequestering the reactive 1,2-dicarbonyls – the important precursors of AGEs. Various AGE inhibitors have been developed and some of them are in advanced clinical trials [43]. Some of the interesting potential drug candidates are: aminoguanidine, metformin, carnosine, homocarnosine, pyridoxamine (Pyridorin®), N-[(2-(hydrazinoiminomethyl)amino)ethyl]acetamide monohydrochloride (ALT-946), 4-oxo-N-phenyl-4,5-dihydro-2-[(1-methylethylidene)hydrazino]-5-thiazoleacetamide (OPB-9195), alagebrium chloride (ALT-711), and N-phenacylthiazolium bromide [43] (Figure 3). Anti-inflammatory drugs such as tenilsetam [3-(2-thienyl)-2-piperazinone] [81] and aspirin [82] also show AGE-inhibiting properties. The antioxidants act as AGE inhibitors, presumably through metal-ion chelation and sequestration of free-radical species.

Much of the early attention for the inhibition of the Maillard reaction was focused on aminoguanidine (Pimagedine®) [9]. Aminoguanidine sequesters toxic 1,2-dicarbonyl compounds through the formation of their corresponding adducts, 1,2,4-triazines, relatively non-toxic compounds. In addition, it has other desirable properties for the attenuation of oxidative stress, such as peroxynitrite scavenging and transition-metal-ion chelation [83]. Through the combination of all of these processes, AGE formation was significantly suppressed. However, the drug is not being further advanced because of adverse side effects that were observed in Phase III clinical trials in patients with diabetes [84]. Some of the adverse side effects could be related to the sequestration of pyridoxal, resulting in vitamin B6 deficiency [85].

Metformin, a bisguanidine, has been used in the treatment of type II diabetes. Recent results suggest that it can reduce blood glucose levels via a serine–threonine protein kinase termed LKB1, which phosphorylates and activates adenosine-monophosphate-activated protein kinase [86]. Metformin is also a moderate AGE inhibitor, because it reacts with methylglyoxal to form the corresponding dihydroimidazolone derivative, although it is not as reactive as aminoguanidine [87].

Pyridoxamine (vitamin B_6, Pyridorin®) and thiamine pyrophosphate have the potential to be at least as effective at AGE inhibition as aminoguanidine [88]. Pyridoxamine has been shown to suppress the formation of the CML and N^{ε}-(carboxyethyl)lysine (CEL), the major products of glycoxidation and lipoxidation reactions, involving the cleavage (or trapping) of glycoxidation- and lipoxidation-derived 1,2-dicarbonyl intermediates [89]. Pyridoxa-

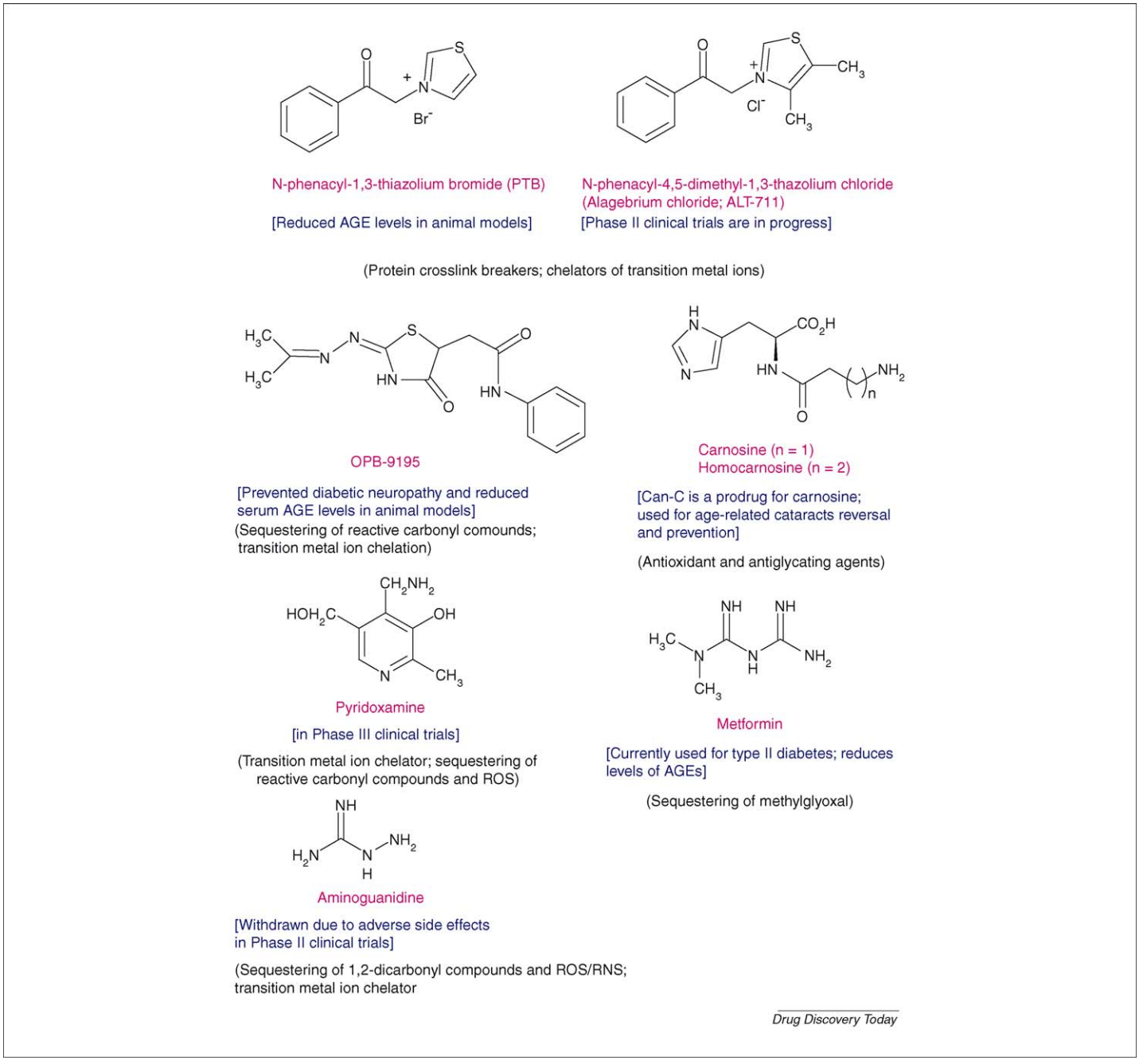

FIGURE 3

Representative advanced glycation end product (AGE) inhibitors and their modes of action. AGE inhibitors can be classified into three classes: (1) carbonyl trapping agents that attenuate carbonyl stress; (2) metal-ion chelators, which suppress glycoxidation reactions; (3) crosslink breakers that reverse AGE crosslinks. Aminoguanidine was historically the first AGE inhibitor explored in clinical trials. However, Phase III clinical trials for aminoguanidine were discontinued because of its side-effects (vitamin B6 deficiency). Pyridoxamine and ALT-711 are promising AGE inhibitors and are currently undergoing clinical trials. Thiazolium salts (OPB-9195 and ALT-711) are AGE crosslink-breakers. Aminoguanidine traps carbonyl compounds. Pyridoxamine, OPB-9195 and carnosine are also metal-ion chelators, thereby attenuating oxidative stress and suppressing the ensuing glycoxidations. Metformin, a commonly used drug for diabetes also acts as an AGE inhibitor.

mine also traps ROS and, thus, inhibits AGE formation by blocking the oxidative degradation of the Amadori intermediates in Maillard reactions [90]. Although it traps reactive carbonyl compounds, it is not as efficient as aminoguanidine for this purpose – pyridoxamine is currently in Phase III clinical trials for the treatment of diabetic nephropathy [91,92].

Carnosine (β-alanyl-L-histidine), a commercially available drug, and its analogues are effective antioxidants because of their ability to chelate transition-metal ions. In addition, they also act as antiglycating agents. However, the AGE-inhibitory action of carnosine is not comparable with that of aminoguanidine. Carnosine protects α-crystallin, superoxide dismutase and catalase against nonenzymatic glycation and protein crosslinking [93], presumably through a combination of its antioxidant and antiglycating properties. Because it is a naturally occurring non-toxic dipeptide, it has potential therapeutic applications as an AGE inhibitor,

perhaps in combination with other AGE inhibitors such as aminoguanidine. N-acetylcarnosine, a prodrug for carnosine, marketed under the trade name Can-C®, is useful for age-related cataract management and prevention, in human and canine eyes [94].

Recently, it has been detected by electrospray ionization tandem mass spectrometry (ESIMS–MS) that carnosine and homocarnosine detoxify the highly reactive aldehyde acrolein, produced by lipid peroxidation, in a phosphate buffer (pH 7.4) system. Acrolein is one of the most reactive and neurotoxic aldehydes and its level is significantly elevated in brains of Alzheimer's disease patients [95]. Potential drugs for Alzheimer's disease (such as desferrioxamine and clioquinol) might have some role as AGE inhibitors because they are effective metal chelators [96]. Iron chelators related to desferrioxamine, perhaps by preventing glycoxidations as a result of reduced oxidative stress, could also offer neuroprotection in other neurodegenerative diseases such as Parkinson's disease [97].

OPB-9195 inhibits AGE formation (especially that of pentosidine and CML) through metal-ion chelation and carbonyl trapping [85]. The apparent binding-constant for copper-ion-chelation for this compound is in the order of 10–100 μM [98]. In animal studies using stroke-prone spontaneously hypertensive rats, it has been shown that OPB-9195 lowers the level of glycated albumin, and reduces blood pressure and oxidative damage [99]. However, it has been discontinued because it depletes vitamin B6. Angiotensin II receptor antagonists (angiotensin II receptor blockers; ARBs) and angiotensin-converting enzyme inhibitor (ACEi) are shown to inhibit AGE formation in *in vitro* studies [85]. These compounds do not trap reactive carbonyls, unlike OPB-9195, instead they strongly chelate transition-metal-ions and sequester ROS [85], thereby inhibiting glycoxidations that are responsible for the AGE formation. Activation of the rennin–angiotensin system contributes to oxidative stress, which might also potentially increase the AGEs [100]. ACEi treatment, using perindopril for human diabetes type 1 cases, resulted in significant increases in plasma sRAGEs [101], which compete with RAGEs in binding to AGEs and attenuate the oxidative stress associated with AGE–RAGE interactions. Clinical trials are currently underway to study the role of the rennin–angiotensin system in the development of diabetes [100]. ACEi and ARB, because of their AGE-lowering properties, could decrease the risk of other AGE-related diseases such as diabetic nephropathy and atherosclerosis [102]. Combination therapies using ACEi and ARBs slow the progression of microalbuminuria to clinically significant albuminuria, showing promise for the prevention and treatment of diabetic nephropathy [103].

AGE inhibition through crosslink breakers

A parallel approach for the attenuation of AGE-related complications would be to reverse the AGE crosslinks. 1,3-Thiazolium derivatives, such as N-phenyl-1,3-thiazolium bromide (PTB) and N-phenacyl-4,5-dimethyl-1,3-thiazolium chloride (alagebrium chloride) (Figure 3), are important protein crosslink breakers [2,98,104], although there are reports of their limited efficacy in *in vivo* studies [105]. Such protein crosslink breakers might be useful as therapeutics for diabetes and related disease complications, and in the prevention of OA [106]. They also act as transition-metal-ion chelators, thus attenuating the oxidative stress leading to the formation of the AGEs. Phenacylthiazolium salts have been investigated in Phase II clinical trials for systolic hypertension [107]. Patients administered with ALT-711 experienced improvement in stiffened cardiovasculature by decreasing pulse-pressure and increasing large-artery compliance [108]. However, the ALT-711 hypertension trials (Phase II) have been terminated because there were no significant changes in blood pressure. Further studies are needed to address the safety issues and efficacy of this drug [109,110]. The mechanism of action of the thiazolium salt-based crosslink breakers is not yet clear, although they seem to be limited to those crosslinks that have 1,2-dicarbonyl residues. Thiazolium salts are among the most potent inhibitors of ascorbate oxidation [98], showing that their antioxidant characteristics can contribute, at least partly, to their AGE-inhibiting properties through the attenuation of glycoxidations.

Conclusions

In vivo Maillard reactions have important consequences in aging and age-related diseases. The tissue concentrations of the AGEs, the end-products of Maillard reactions, are correlated with the progression of diabetes and related vascular complications. Further, AGEs are associated with various age-related pathologies such as Alzheimer's disease, Parkinson's disease, and ALS. Consequently, development of AGE inhibitors and AGE breakers as therapeutics has been attracting renewed attention in recent years. In view of the adverse side-effects associated with many of the potent AGE inhibitors and/or AGE breakers, it is apparent that naturally occurring antiglycating and/or anti-inflammatory agents (such as pyridoxamine and carnosine, and their derivatives) can offer practical approaches for the prevention and/or treatment of AGE-associated diseases. Modified versions of thiazolium salts and aminoguanidine (that do not cause vitamin B6 deficiency) are attractive targets in this area. It is hoped that combination therapy using the AGE inhibitors, or their supplementation with other antioxidants, would serve as a common strategy in the treatment of various AGE-mediated pathologies.

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