

Advanced glycation endproducts (AGEs): pharmacological inhibition in diabetes

Les produits de Maillard (ou AGEs) : leur inhibition pharmacologique au cours du diabète

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Available online 15 September 2006

Abstract

AGE inhibitors may act by various mechanisms at different steps of advanced glycation endproduct (AGE) formation (depending on oxidative stress and/or carbonyl stress) and AGE-mediated damage: trapping of reactive dicarbonyl species; antioxidant activity by transition metal chelation; other antioxidant activity including free radical scavenging; AGE cross-link breaking; AGE receptor (RAGE) blocking; RAGE signaling blocking; glycemia reduction by anti-diabetic therapy; aldose reductase inhibition; shunting of trioses-P towards the pentose-P pathway by transketolase activation. Most of the inhibitors have several sites of action. Practically one can distinguish drugs specifically developed as AGE inhibitors or AGE breakers; RAGE and receptor signaling blockers; other therapeutic compounds which were found subsequently to possess also AGE inhibitor activity, including dietary antioxidants. Encouraging results obtained in studies of various AGE inhibitors, conducted in vitro and in diabetic animals, are summarized in this review. However most of the clinical trials have been more or less disappointing, in part because of side effects; the long-term therapeutic interest of the most recently developed AGE inhibitors or breakers remains to be demonstrated in diabetes.

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Résumé

Les inhibiteurs d'AGE peuvent agir par divers mécanismes aux différentes étapes de formation des AGE (liées au stress oxydant et/ou au stress carbonyle) et de leur effets biologiques : capture des intermédiaires dicarbonylés réactifs ; activité antioxydante par chélation de métaux de transition ; autre activité antioxydante, y compris le piégeage des radicaux libres ; clivage des liaisons croisées des AGE ; inhibition des récepteurs des AGE (RAGE) ; blocage des voies de signalisation des RAGE ; réduction de la glycémie par les antidiabétiques ; inhibition de l'aldose réductase ; court-circuitage des trioses-P vers la voie des pentoses-P par activation de la transcétolase. La plupart des inhibiteurs des AGE ont plusieurs sites d'action. En pratique on peut distinguer les drogues développées spécifiquement comme inhibiteurs d'AGE ou cliveurs d'AGE ; les inhibiteurs du RAGE et de ses voies de signalisation ; d'autres composés utilisés en thérapeutique et qui se sont révélés posséder également des propriétés inhibitrices des AGE, auxquelles on peut rattacher des antioxydants naturels alimentaires. Cette revue présente les résultats obtenus in vitro et chez l'animal diabétique qui montrent l'intérêt potentiel des inhibiteurs d'AGE. Cependant, la plupart des essais cliniques ont été plus ou moins décevants, en partie à cause d'effets secondaires ; l'intérêt thérapeutique à long terme des inhibiteurs ou des cliveurs d'AGE les plus récents reste encore à établir.

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Keywords: AGE inhibitors; AGE breakers; Aminoguanidine; OPB 9195; LR compounds; Pyridoxamine; Benfotiamine; ALT 711; sRAGE; Metformin; ACE inhibitors; Angiotensin II receptor antagonists; Aldose reductase inhibitors; Diclophenac; Salicylates; Tenilsetam; Flavonoids

Mots clés : Inhibiteurs d'AGE ; Agents cliveurs d'AGE ; Aminoguanidine ; OPB 9195 ; Composés LR ; Pyridoxamine ; Benfotiamine ; ALT 711 ; sRAGE ; Metformine ; Inhibiteurs de l'enzyme de conversion de l'angiotensine ; Antagonistes des récepteurs de l'angiotensine II ; Inhibiteurs de l'aldose réductase ; Diclophenac ; Salicylates ; Tenilsetam ; Flavonoïdes

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1. Introduction

The advanced glycation endproducts (AGEs) play an important role in the development of chronic diabetic complications [1]. Therefore inhibiting their formation and/or their deleterious effects has been searched for as well as increasing their breakdown and/or elimination. After a short review of AGE formation and their effects on extracellular matrices and cells, we shall consider different approaches of their pharmacological inhibition.

2. AGE formation

The Maillard reaction products described by Louis Camille Maillard in 1912 [2] were initially appreciated during food preparation and heating when reducing sugars reacted with aminogroups of aminoacids or proteins to produce flavorful and brownish compounds also called AGEs. These exogenous dietary AGEs were first studied in nutritional chemistry. When HbA_{1c} was discovered in diabetic patients, the importance of the glycation reaction (glycosylation without enzymatic intervention) and its advanced endogenous endproducts started to be investigated. Galactose, fructose and ribose reacted quicker than glucose with hemoglobin [3]. The classical pathway leading to AGEs through Amadori products such as HbA_{1c} was described (Fig. 1). Protein glycation is initiated by addition reaction between a free aminogroup and the carbonyl group of a sugar to form a reversible Schiff base (in a period of hours). The latter can rearrange into a stable ketoamine or Amadori product (over a period of days). *Protein glycation is a spontaneous reaction depending in vivo on the degree and duration of hyperglycemia.* The Amadori product can be trans-

formed (in a period of weeks) into *reactive dicarbonyl products* such as glucosones (for instance 1,4-deoxyglucosone, precursor of glucosepane) to form AGEs, such as glucosepane (Fig. 2). The Amadori product can also be fragmented by oxidation (*glycooxidation*) to produce AGEs like carboxymethyl-lysine (CML) or pentosidine [4] (Figs. 2 and 3). AGEs accumulate mainly in proteins with long half-life, such as *extracellular matrix collagens*. Recently an enzyme degrading Amadori products has been described, fructosamine 3-kinase.

Other pathways for AGE formation have been described (Fig. 3). Glucose can be directly oxidized in the presence of catalytic metals and O₂ (*autooxidation*) [1,4] (Figs. 3 and 4). Metal-catalyzed glucose autooxidation may be important in diabetes with renal insufficiency or/and atherosclerosis [4]. It leads to the formation of glyoxal (CHO–CHO) and arabinose. The latter pentose may react with proteins to form AGEs such as pentosidine (Fig. 2).

Methylglyoxal (CH₃-CO-::CHO) has been identified as a major *intracellular* reactive dicarbonyl intermediate originating from glycolysis: spontaneous dephosphorylation of glyceraldehyde-3-P and dihydroxyacetone-P at the triose-P isomerase step results in methylglyoxal formation. In addition, ascorbic acid, threonine and aminoacetone are methylglyoxal precursors (Fig. 3). Aminoacetone is oxidized by semicarbazide-sensitive amino-oxidase (SSAO) into methylglyoxal and H₂O₂. Methylglyoxal may be catabolized by intracellular glyoxalases. Methylglyoxal reacts with free lysine groups to make AGEs such as carboxyethyl-lysine (CEL) or methylglyoxal lysine dimer (MOLD) (Figs. 2 and 3). It reacts also with free arginine groups to make hydroimidazolones. Methylglyoxal may be catabolized by intracellular glyoxalases.

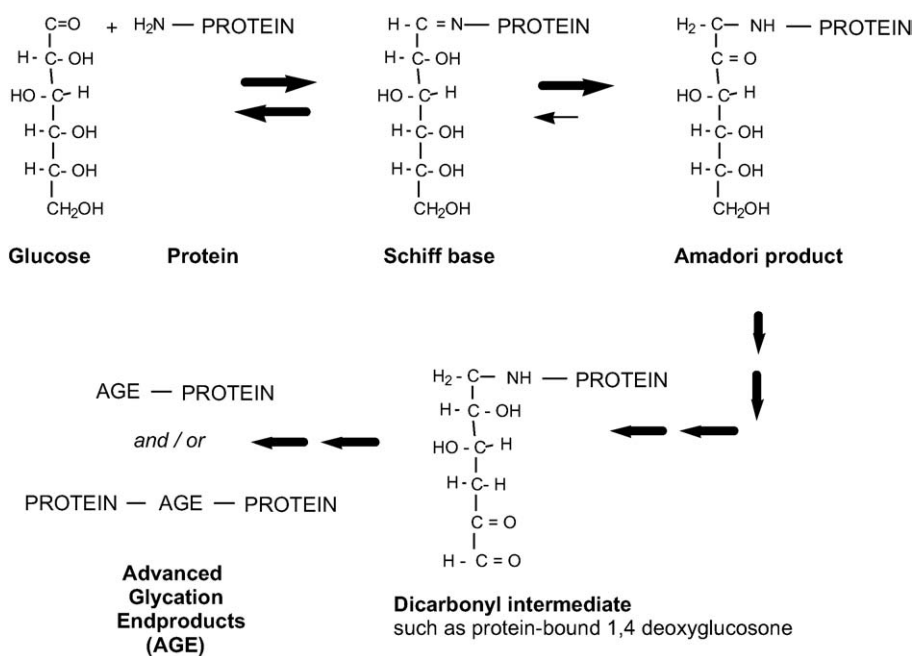


Fig. 1. Example of the classical pathway of protein glycation by glucose leading to AGEs via Amadori products. The initial reaction between glucose and protein amino group forms a reversible Schiff base which rearranges to a fructosamine group or Amadori product. With time Amadori products may form AGEs via dicarbonyl intermediates such as protein-bound 1,4 deoxyglucosone.

Inside cells with free non-insulin-dependent glucose transport, such as endothelial cells, *increased flux from glucose to pyruvate and NADH into mitochondria* leads to increased mito-

chondrial electron leakage and reduction of oxygen into superoxide anion, according to the “unifying theory of diabetic complications” [7]. Superoxide anion is transformed by superoxide dismutase into H_2O_2 . This would result in *oxidant damage* to glyceraldehyde 3-P dehydrogenase and activation of deleterious pathways upstream of this enzyme: *methylglyoxal derived AGE formation*, activation of protein kinase C, activation of the hexosamine pathway leading to increased gene transcription such as PAI-1, and *increased aldose reductase (AR) activity which favors methylglyoxal and subsequent AGE production* (Fig. 4).

Oxidation of polyunsaturated fatty acids (*lipoxidation*) can also lead to glyoxal or methylglyoxal, apart from the characteristic advanced lipoxidation endproducts (ALEs) such as malondialdehyde or 4-hydroxynonenal. Glyoxal reacts with free lysine groups to make CML or glyoxal lysine dimer (GOLD) (Figs. 2 and 3). Glyoxal reacts with free arginine groups to make hydroimidazolones.

Therefore some AGEs such as CML and CEL are *endproducts of both glycoxidation and lipoxidation pathways*. In contrast other AGEs such as pentosidine are produced *only by glycoxidation*.

According to their chemical structure, three types of AGEs may be distinguished (Fig. 2): (A) fluorescent cross-linking AGEs such as pentosidine and crossline, (B) non-fluorescent cross-linking AGEs such as glucosepane and MOLD, (C) non-cross-linking AGEs (or adducts) such as CML and pyrraline.

3. Harmful effects of AGEs

The cross-linking AGEs alter the physico-chemical properties of proteins (particularly those with long half-life) and may

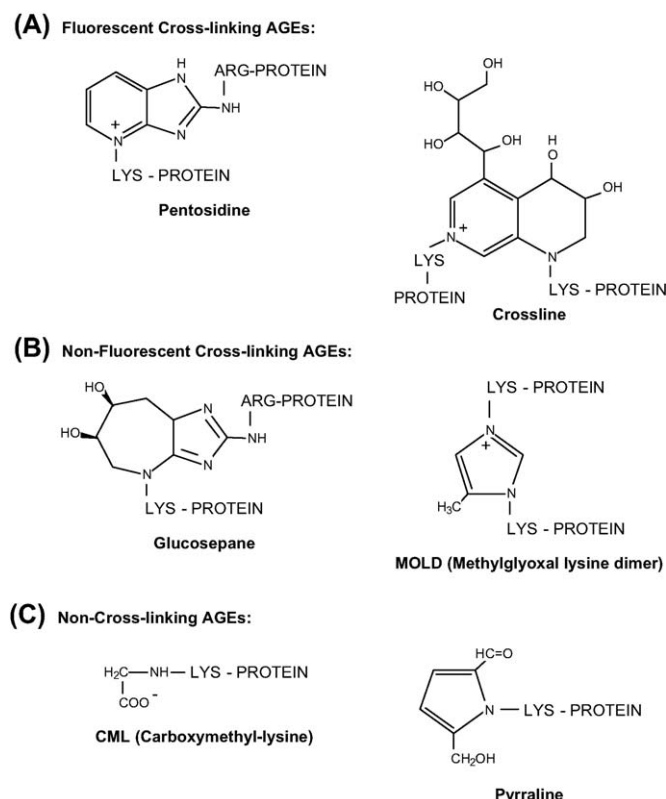


Fig. 2. Chemical structures of three types of AGEs: (A) fluorescent cross-linking AGEs such as pentosidine and crossline, (B) non-fluorescent cross-linking AGEs such as glucosepane and MOLD, (C) non-cross-linking AGEs (or adducts) such as CML and pyrraline.

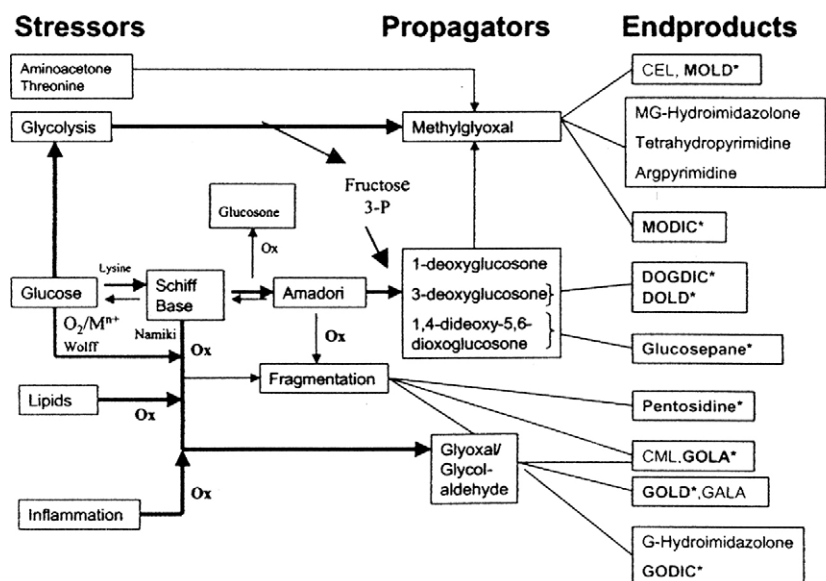


Fig. 3. Main chemical pathways leading to AGE formation and selected AGEs of relevance to the Maillard reaction in vivo (from [4], with permission). * denotes a crosslink.

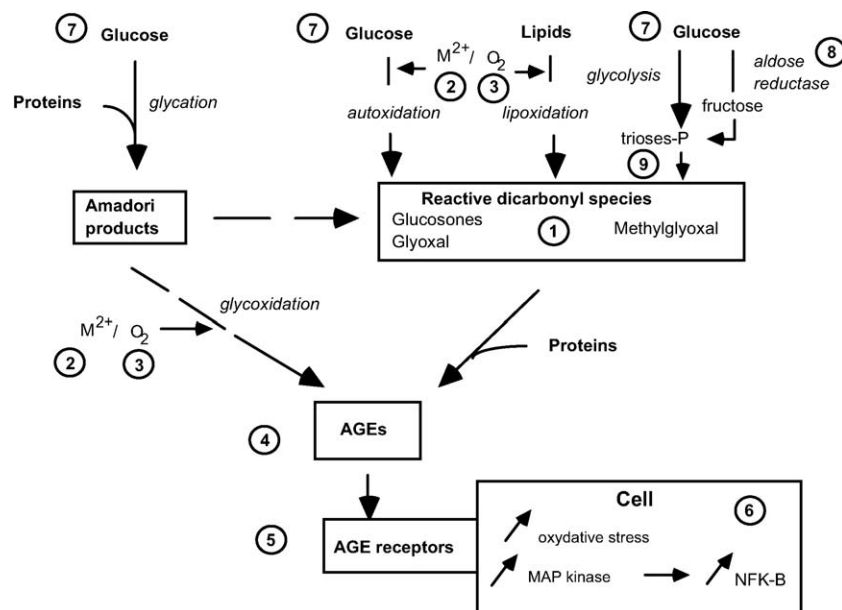


Fig. 4. Potential sites of inhibition of AGE formation and AGE-mediated damage: (1) trapping of reactive dicarbonyl species; (2) antioxidant activity by transition metal (M^{2+}) chelation; (3) other antioxidant activity including free radical scavenging; (4) AGE cross-link cleavage (by AGE breakers); (5) AGE receptor (RAGE) blocking; (6) AGE receptor (RAGE) signaling blocking; (7) glycemia reduction by anti-diabetic therapy; (8) aldose reductase inhibition; (9) shunting of trioses-P towards the pentose-P pathway by transketolase activation. Excessive reactions of protein amino-groups with reactive dicarbonyl species are characteristic of *carbonyl stress*. Glycoxidation, glucose autooxidation and lipoxidation contribute to *oxidant stress*.

thus influence their functional properties [5]. This is the case, for instance, for insoluble long-lived glomerular basement membrane (GBM) proteins and other *extracellular matrix proteins* [6].

Circulating AGEs may bind to cell membrane receptors and elicit intracellular damage. Cells possess different receptors for AGEs: macrophage scavenger receptors type I and II, galectin-3 (AGE-R3), oligosaccharyl transferase-48 (AGE-R2), but the best studied is the receptor for AGEs (RAGE). RAGE can be stimulated by CML and other AGEs, but also different ligands including S-100 calgranulins which are a group of pro-inflammatory cytokines, amphoterin, amyloid- β and other fibrillar proteins. Expression of RAGE is enhanced in certain cells during diabetes and inflammation. Interaction of AGEs with RAGE on the membrane of cells such as macrophages, mesangial or endothelial cells, pericytes, causes intracellular oxidative stress and activation of nuclear factor NF- κ B via activation of the mitogen-activated protein (MAP) kinase signaling pathway (Fig. 4). NF- κ B modulates gene transcription for various factors: endothelin-1, vascular endothelial growth factor (VEGF), transforming growth factor β (TGF- β), pro-inflammatory cytokines such as interleukins IL-1 α , IL-6 and tumor necrosis factor α (TNF- α). There is also enhanced expression of adhesion molecules such as vascular cell adhesion molecule VCAM-1 and intercellular adhesion molecule (ICAM-1), of extracellular proteins like laminin and type IV collagen, in addition to other effects such as increased vascular permeability or angiogenesis.

AGEs have been shown to be implicated in the development of diabetic complications [1,8]. But the relative importance of their role is still discussed. Moreover not only endogenous

AGEs seem to be pathogenic, but exogenous dietary AGEs from cooked food, recovered in the circulation, might also contribute to some extent to the development of diabetic complications [9].

4. Pharmacological inhibition of AGEs

Intervention against the Maillard reaction in vivo may be situated at different steps of AGE formation and AGE-mediated damage (Fig. 4):

- trapping of reactive dicarbonyl species;
- antioxidant activity by transition metal chelation;
- other antioxidant activity including free radical scavenging;
- AGE cross-link cleavage (by AGE breakers);
- AGE receptor blocking;
- AGE receptor signaling blocking;
- glycemia reduction by anti-diabetic therapy;
- AR inhibition;
- shunting of trioses-P towards the pentose-P pathway by transketolase activation.

Most of the inhibitors have several sites of action. Practically, one can distinguish: drugs specifically developed as AGE inhibitors or AGE breakers; RAGE and receptor signaling blockers; other therapeutic compounds which were found subsequently to possess also AGE inhibitor activity, including dietary antioxidants.

4.1. Drugs specifically developed as AGE inhibitors or AGE breakers (Table 1)

4.1.1. Aminoguanidine (AMG)

The possibility to prevent the formation of AGEs was reported for the first time by Brownlee et al. [10] with AMG. AMG prevented formation of fluorescent AGEs and glucose-derived collagen cross-links in vitro. AMG administration to diabetic rats also prevented formation of fluorescent AGEs and cross-linking of arterial wall connective tissue proteins. Since then, the use of AMG to prevent AGE formation in vitro and in vivo has given evidence of the involvement of AGEs in pathological states and in aging.

In model systems, the mechanism by which AMG inhibits AGEs formation has been shown to involve *trapping of reactive dicarbonyl intermediates* [11,12]. At high concentration, there is also significant reaction with other carbonyl compounds. AMG can react with pyruvate under physiological conditions to form a hydrazone adduct [12]. Similarly, there is a slow reaction with glucose to form β -D glucopyranosyl AMG-adduct [13,14]. Furthermore, AMG is an *inhibitor of nitric oxide synthase* (NOS), more potent on inducible form of NOS (iNOS; IC₅₀ = 31 μ M) than on neuronal NOS (nNOS; IC₅₀ = 170 μ M) and endothelial NOS (eNOS; IC₅₀ = 330 μ M) [15]. Since the IC₅₀ for inhibition of protein glycation by methyl glyoxal in human plasma is 203 μ M [16], it is likely that in all cases where AMG is used to prevent glycation reaction, it is also competent to inhibit iNOS and probably nNOS [12]. AMG is also a potent and irreversible *inhibitor of SSAO* which catalyses the conversion of aminoacetone to methylglyoxal [17]. In retinal Muller cells, AMG has been found to act as an *antioxidant*, quenching hydroxyl radicals and lipid peroxidation [18]. A decrease in lipid peroxidation is also observed in vivo in streptozotocin (STZ) diabetic rats treated by AMG (1 g/l in drinking water) for 9 weeks [19] and in diabetic rabbits (400 mg/l in drinking water for 10 months) [18]. Price et al. [20] proposed that AMG and many other AGE inhibitors at millimolar concentration, act primarily by chelating or antioxidant activity rather than carbonyl-trapping activity. But triazine adducts of carbonyl compounds were identified during glycooxidation and lipoxidation reactions in vivo [11] confirming that in addition to its chelating and antioxidant activity AMG also acts as a true scavenger of carbonyl compounds.

AMG effects on experimental diabetic nephropathy – In STZ-diabetic rats, administration of AMG (1 g/l in drinking water) for 32 weeks, attenuated albuminuria and prevented mesangial expansion. There was a concomitant decrease in glomerular and tubular fluorescence and AGE content evaluated with anti-AGE antibodies [21,22]. In the same model, AMG has been found to attenuate overexpression of TGF- β 1 and PDGF- β and to reduce type IV collagen deposition in glomeruli [23]. AMG reduced the expression of TNF- α and iNOS in glomeruli of STZ-diabetic rats at 52 weeks of diabetes [24]. However, when comparing the effects of AMG with the effects of a NOS inhibitor, L-NAME, in STZ-diabetic rats, Soulis et

al. [25] found that AMG but not L-NAME could prevent albuminuria and renal AGE levels. This suggests that the effect of AMG is mediated predominantly by AGE formation inhibition. In STZ-diabetic rats, AMG prevented increases in PKC activity in the glomeruli, retina and mesenteric artery, in parallel with suppression of albuminuria [26]. However, in STZ-diabetic baboons, AMG (10 m/kg) given subcutaneously daily over a period of 4 years prevented GBM thickening, but was not able to prevent albuminuria [27]. In Otsuka Long Evans Tokushima fatty (OLEFT) rats (a model of type 2 diabetes), AMG (1 g/l in drinking water for 40 weeks) prevented development of albuminuria, mesangial expansion and GBM thickening [28].

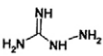
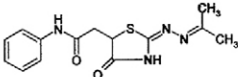
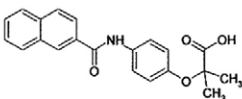
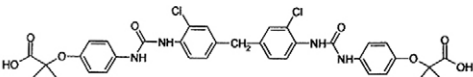
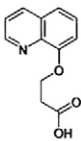
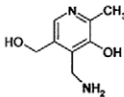
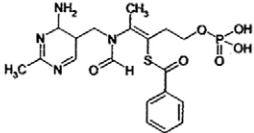
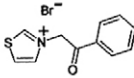
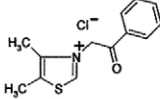
AMG effects on experimental diabetic retinopathy – Treatment of STZ-diabetic rats for 26 weeks with AMG (25 mg/kg IP daily) prevented accumulation of AGEs at branching sites of precapillary arterioles and abnormal endothelial cell proliferation; pericyte loss was diminished. Under the same experimental conditions, treatment with AMG (0.5 g/l in drinking water) for 75 weeks also decreased the acellular capillaries and microaneurysms [29]. In another experiment in diabetic rats treated with AMG (0.5 g/l), acellular capillaries increased over the first 24 weeks of treatment and then stopped increasing for the rest of the study (52 weeks), suggesting that AMG does not inhibit the initial phase of experimental diabetic retinopathy in rats [30]. In diabetic rats treated either with AMG, an AGE- and NOS inhibitor, or L-NAME, a NOS inhibitor, and 2,3-diamino-phenazine, an AGE- but not NOS inhibitor, Roufai et al. [31] have observed that only AMG and 2,3-diamino-phenazine prevented depletion in nNOS-containing neurons, characteristic of diabetic retinopathy suggesting that this effect is mediated by the inhibition of AGE formation. However, in alloxan-diabetic rats, AMG (0.5 g/l in drinking water for 32 weeks) can inhibit accelerated death of retinal capillary cells and development of retinopathy without modifying AGE-hemoglobin levels or tail collagen pentosidine level, fluorescence intensity and thermal breaking time [32]. Same results were observed in a 5-year study in diabetic dogs: AMG (200 mg/kg daily in tablets) inhibited development of retinal microaneurysms and acellular capillaries, but had no influence on pentosidine levels in tail collagen and aorta [33].

AMG effects on experimental diabetic neuropathy – In STZ-diabetic rats, AMG administration (25 or 50 mg daily) has been found to normalize reduction in sciatic nerve blood flow and to improve conduction, in a dose-dependent manner [34]. In similar experiments, AMG was found to improve motor nerve conduction velocity [35,36] and to inhibit accumulation of fluorescent AGEs in nerves [35]. The effects of AMG on nerve conduction velocity were completely blocked by co-treatment with NG-nitro-L-arginine (a NOS inhibitor) suggesting a neurovascular mechanism for AMG effects involving improved NO activity due to decreased oxidative stress [37]. In STZ-diabetic rats, AMG did not influence neuroaxonal dystrophy, a feature of autonomic neuropathy [38]. AMG (10 mg/kg daily SC) did not restore conduction velocity or autonomic dysfunction over a 5-year period in diabetic baboons [39].

AMG effects on aging process – Aging is characterized by structural and functional changes of the cardiovascular and

Table 1

Drugs specifically developed as AGE inhibitors or AGE breakers

Generic name	Structure
I- AGE inhibitors	
- Guanidine structure Aminoguanidine (Pimagedine)	
- Thiazolidine structure OPB-91295	
- Ureido/carboxamidophenoxy-isobutyric acids LR-9	
LR-90	
- Quinoloxiny propionic acid LR-74	
- Vitamins Pyridoxamine (Pyridorin)	
Benfotiamine	
II- AGE breakers	
- N-phenacyl thiazolium structure PTB	
ALT-711 (Alagebrium)	

renal systems. It has been proposed that most of these cardiovascular and renal modifications were related to glycation of proteins and production of AGEs [40]. Fischer 344 and Sprague Dawley rats treated from 6 to 24 months of age by AMG (1 g/l in drinking water) had a reduced content of AGEs in plasma, heart, blood vessels and kidneys; the age-related cardiac hypertrophy and the decrease in endothelial-dependent vasodilatory response reported in the control aging rats were prevented in the older animals. The treatment also reduced proteinuria and glomerular sclerosis without modification of GBM thickness [41]. AMG administration in drinking water (1 g/l) to Fisher 344 rats from 24 to 30 months of age prevented the end-life increase in arterial stiffness and cardiac hypertrophy without any change in total collagen and elastin content of the arterial wall suggesting a reduction in the AGE-induced cross-

linking of heart and arterial wall extra cellular matrix proteins [42]. In a similar experiment, AMG did not affect collagen glycation or glycooxidation, except for a modest decrease in tail tendon break time [43].

Clinical trials were designed to evaluate the safety and efficacy of AMG in retarding the rate of progression of renal disease in patients with overt diabetic nephropathy. Two phase III clinical trials were conducted and completed in 1998: ACTION I with type 1 diabetic patients and ACTION II with type 2 diabetic patients. ACTION I was a randomized, double blind, placebo-controlled trial comparing two dose levels of AMG (150 and 300 mg daily) with placebo on the progression of nephropathy in 690 patients. The primary end point was the time to doubling of serum creatinine. The secondary end point included evaluation of proteinuria, kidney function and

retinopathy. AMG therapy lowered LDL and triglycerides and increased HDL with the low dose but not with the high dose. In the combined dose group, a reduced progression of retinopathy and a non-significant tendency towards slower serum creatinine doubling time were observed [44]. ACTION II was performed in 559 patients with similar protocol and end points [45] but due to safety concerns and apparent lack of efficacy, the study was discontinued. Although beneficial effects of AMG against diabetic complications have been widely confirmed in animal models, AMG did not achieve successful clinical trials. This may be attributed to its rapid renal clearance, its moderate dicarbonyl scavenging at pharmacological concentrations in vivo and its toxicity [12].

4.1.2. OPB-9195

OPB-9195 is a hydrazine derivative, as AMG. In vitro, it inhibits pentosidine generation from diabetic and uremic plasma [46]. It also inhibits, in a dose-dependent manner, formation of pentosidine and CML from a variety of individual precursors including ribose, glucose and ascorbate as well as formation of two ALEs, malondialdehyde-lysine and 4-hydroxynonenal from arachidonate [47]. Its mechanism of action appears similar to that proposed for AMG. OPB-9195 traps dicarbonyl intermediates of advanced glycation more efficiently than AMG [46]. Moreover its chelating activity is much more efficient than that of AMG or pyridoxamine (PM) [20].

In vivo, OPB-9195 prevented several biological effects associated with AGE formation. In OLETF rats, it prevented progression of *glomerular sclerosis* and AGE deposition in glomeruli. In addition, circulating AGE levels and albumin excretion were highly decreased in spite of persistent hyperglycemia [48]. Blockade of type IV collagen production and overproduction of TGF- β and VEGF seems to be involved in the renal protective effects of OPB-9195 [49]. In RAGE overexpressing mice, oral administration of OPB-9195 for 5 months reduced serum AGE levels and prevented glomerular sclerosis [50]. In STZ-diabetic rats, OPB-9195 (60 mg/kg given by daily gavages for 24 weeks) improved tibial *motor nerve conduction velocity* and restored sciatic nerve Na⁺K⁺ATPase activity. Expression of immunoreactive AGEs in the sciatic nerve was reduced [51]. Recently, long-term administration of OPB-9195 (36 weeks) has been found to prevent retinal microvascular cell apoptosis in Goto-Kakizaki rats, a type 2 diabetic model [52].

Unfortunately, the *clinical trials* of this compound given to diabetic patients were hampered, as for AMG, by side effects related to the characteristic trapping of pyridoxal, resulting in vitamin B6 deficiency syndrome [53].

4.1.3. LR compounds

Recently, two new classes of aromatic compounds, derivatives of aryl (and heterocyclic) ureido- and aryl (and heterocyclic) carboxamido- phenoxy-isobutyric acids and benzoic acids, have been reported to be potential inhibitors of glycation and AGE formation [54]. Three of these compounds prevented the development of diabetic nephropathy: LR-90, LR-9 and LR-74.

The mechanism of action of these compounds is not yet well determined. In vitro studies showed that they could directly interact with several *reactive dicarbonyl species* such as glyoxal, methylglyoxal and glycolaldehyde. They inhibited post-Amadori AGE formation more efficiently than PM. They were found to be *potent chelators* of Cu²⁺ with IC₅₀'s of 50–275 μ M; this probably explains that they can suppress hydroxyl radical production during sugar autooxidation and glycation reactions [54].

In vivo, LR-90 treatment (50 mg/l in drinking water) of STZ-diabetic rats for 32 weeks inhibited increase in albuminuria and serum creatinine level. LR-90 prevented glomerulosclerosis, tubular degeneration and collagen deposition in the kidney. It also decreased AGE accumulation in kidney glomeruli and nitrotyrosine deposition in renal cortex. Interestingly, LR-90-treated diabetic rats showed higher body weight than untreated diabetic controls [55].

LR-9 or LR-74 (50 mg/l in drinking water for 32 weeks) also inhibited albuminuria and plasma creatinine increase in STZ-diabetic rats. They reduced CML accumulation in kidney glomeruli and tubules, AGE-linked fluorescence and cross-linking of tail collagen, CML and CEL content of skin collagen. Additionally, they lowered plasma cholesterol and triglycerides levels and inhibited lipid peroxidation [56].

4.1.4. Pyridoxamine

PM is one of the three natural forms of vitamin B6. In vitro, PM unlike AMG strongly inhibited CML formation from isolated Amadori products [57]. PM does not interact directly with Amadori intermediates, but interferes with the post-Amadori oxidative reactions by binding catalytic redox metal ions [58]. PM also traps reactive low molecular weight carbonyl compounds derived from either sugars or lipids, inhibiting AGE and ALE adducts [59,60]. Moreover, adducts of PM deriving from catabolites of arachidonic and linoleic acids were detected in urine of PM-treated diabetic, hyperlipemic and control rats indicating that carbonyl-trapping mechanism is operative in vivo [61].

In STZ-diabetic rats, Degenhard et al. [62] showed that, at comparable doses (1 g/l in drinking water for 7 months), PM was superior to AMG in retarding the development of *renal disease* as measured by albuminuria and plasma creatinine. PM caused a partial correction of the increase in glomerular volume without significant effects on GBM and mesangial volume. PM corrected hyperlipidemia and plasma lactate/pyruvate ratio without modifying blood glucose and HbA_{1c} levels; in skin collagen, PM decreased cross-linking, fluorescence and CML and CEL (but not pentosidine) levels. In a similar model, PM prevented *retinal vascular alterations*: capillary loss, laminin upregulation and CML accumulation [63].

Zucker obese (fa/fa) rats are characterized by obesity, hyperlipidemia, mild hypertension and insulin resistance. Despite the fact that they are normoglycemic, increases in CML, CEL, pentosidine and fluorescence in the skin collagen are similar to those observed in STZ-diabetic rats. PM (2 g/l in drinking water) decreased hypertension and vascular wall

thickening, CML and CEL levels in skin collagen, triglycerides, cholesterol, and creatinine plasma levels and nearly normalized albuminuria [64]. These results, observed in hyperlipidemic non-hyperglycemic rats, show that lipids may be as responsible as carbohydrate for the chemical modifications of proteins and the development of complications in diabetes.

In type 1 diabetic patients, PM showed a favorable safety profile. Phase II trials are ongoing to evaluate the efficacy of PM in inhibiting the progression of proteinuria and hyperlipidemia in diabetic patients with early stage kidney disease [65].

4.1.5. Benfotiamine

Benfotiamine is a lipophilic derivative of thiamine (vitamin B1) designed to be a better *activator of transketolase* (converting glyceraldehyde-3-phosphate into pentose-5-phosphate) than thiamine itself. Thus, its proposed mechanism of action involves shunting of triose glycolytic intermediates towards the reductive pentose pathway [69]. Indeed, in human endothelial cells and bovine retinal pericytes cultured in high glucose, benfotiamine increased transketolase expression or activity, but also reduced aldose reductase mRNA expression and intracellular glucose and sorbitol levels [66].

Administration of benfotiamine for 9 months completely prevented hexosamine and PKC activation, intracellular AGE formation and appearance of acellular capillaries in retina of diabetic rats [67]. In STZ-diabetic rats, preventive administration of benfotiamine (but not thiamine) for 3 months increased motor nerve conduction velocity and inhibited neural imidazole-type AGE and CML accumulation [68]. Oral administration of benfotiamine at high dose (70 mg/kg daily) to STZ-diabetic rats increased transketolase expression in renal glomeruli and conversion of triose phosphate to ribose-5 phosphate; it decreased the levels of different AGEs, microalbuminuria and PKC activation [69]. Recently, benfotiamine was shown to accelerate healing of ischemic limbs in diabetic mice. It increased muscular transketolase activity, prevented ischemia-induced toe necrosis, and improved hind limb perfusion and endothelium-dependent vasodilatation. In addition benfotiamine prevented vascular accumulation of AGEs and induction of proapoptotic caspase-3 while restoring proper expression of eNOS and protein kinase B (PKB) in ischemic muscle. Increased PKB activity seems to play an important role since the benefits of benfotiamine are nullified by dominant-negative PKB construct [70].

4.1.6. AGE breakers: PTB and ALT-711

AGE breakers have been shown to cleave AGE-protein cross-links by some tests in vitro. However their beneficial effects observed in diabetic animals or in patients are not necessarily or exclusively due to a cleaving mechanism, which is very difficult to demonstrate in vivo.

Vasan et al. [71] showed for the first time that it was possible to break established glucose-derived AGE-protein cross-links by pharmacological agents: *N*-phenacyl thiazolinium bromide (PTB) was reported to cleave AGE cross-link between albumin and collagen and reduce the amount of immunoglobu-

lin bound to circulating red blood cells. In STZ-diabetic rats, PTB could prevent vascular AGE accumulation [72]. However, other studies showed that, although PTB could reduce AGE cross-links in vitro, it did not cleave AGE cross-links formed in skin collagen of diabetic rats [73,74]. Because of the instable nature of PTB in physiological buffers [75], several analogs have been developed. *N*-phenacyl-4,5-dimethylthiazolium chloride (ALT-711) has been studied, almost exclusively.

ALT-711 has been claimed to catalytically break established AGE cross-links between proteins. However some investigators attributed the pharmacological effects of ALT-711 to inhibition of AGE formation, in addition to its effects on AGE cross-links. Yang et al. [74] reported that ALT-711 did not improve the solubility of tail tendon and skin collagen isolated from 7 months-diabetic rats and incubated with ALT-711. But a treatment of STZ-diabetic rats with ALT-711 initiated after 2 months of diabetes normalized large artery stiffness assessed by characteristic input impedance and systemic arterial compliance; however, no difference in cross-linking between controls and treated animals could be observed with the use of differential scanning calorimetry [76]. As ALT-711 and its hydrolytic products have been shown to be among the most potent inhibitors of copper-catalyzed oxidation of ascorbate, it has been proposed that ALT-711 might act in vivo by preventing metal-catalyzed glycoxidation [20].

Regardless of its mechanism of action, the results of experimental and clinical studies have indicated the potential utility of ALT-711 in reversing the complications of aging and diabetes. In STZ-diabetic rats, curative administration of ALT-711 (1 mg/kg daily, IP, for 1–3 weeks) reversed the increase in large artery stiffness as measured by systemic arterial compliance, aortic impedance and distensibility [77]. Oral administration of ALT-711 (1 mg/kg daily) for 1 month to aged dogs resulted in improvement of end-diastolic and stroke volume indices and decrease in left ventricular stiffness [78]. In aged monkeys treated with ALT-711 (1 mg/kg, IM, daily for 11 days) a prolonged decrease in pulse wave velocity and aortic stiffness was observed in comparison with the pre-treatment values [79]. Other studies examined the combined effects of aging and diabetes or hypertension on left ventricular function and myocardial collagen content. Mongrel dogs, 9–12-year-old at the beginning of the experiment, were fed with ALT-711 (1 mg/kg daily for 1 month) 5 months after the induction of diabetes with alloxan. The treatment restored left ventricular ejection fraction, reduced left ventricular mass and aortic stiffness and reversed myocardial collagen types I and III accumulation. ALT-711 also increased left ventricular collagen solubility [80]. In 45-week-old spontaneous hypertensive rats (SHR), oral administration of ALT-711 (1 mg/kg daily for 4 months) reduced left ventricular and aortic mass indices and left ventricular hydroxyproline concentrations. In older SHR, ALT-711 reduced systolic pressure and albuminuria [81].

In STZ-diabetic rats, oral administration of ALT-711 (10 mg/kg) from the 16th to the 32nd week of diabetes resulted in a significant decrease in renal CML and RAGE immunostaining, albumin excretion rate, gene expression of TGF- β 1, connective tissue growth factor (CTGF) and type IV collagen

[82]; the *renoprotective effects* of ALT 711 might, in part, occur via PKC- α inhibition in the renal cortex [83]. In a model of diabetes associated with atherosclerosis, the STZ-diabetic and apolipoprotein-E-deficient mouse, preventive ALT-711 treatment (20 mg/kg, daily for 20 weeks) reduced albuminuria, renal RAGE and AGE R-2 gene expression and glomerular accumulation of type I and type IV collagens without modifying triglycerides and HbA_{1c} levels [84]. In the same model, ALT-711 (10 mg/kg, daily for 20 weeks) reduced atherosclerotic plaque areas in thoracic and abdominal regions, but not in aortic arch region, and attenuated the overall increase in plaque collagen content with a more profound reduction in type IV collagen than in type III collagen and no effect on type I collagen; this was accompanied by a reduction in RAGE expression and CML content in aorta [85].

Phase II clinical trials of ALT-711 were initiated in 1998. Effects of ALT-711 on blood pressure and vascular elasticity were tested by Kass et al. in 93 individuals over the age of 50 with evidence of vascular stiffening. The patients who received ALT 711 (210 mg/day, for 8 weeks) showed reduction in arterial pulse pressure and increase in large artery compliance. ALT-711 was well tolerated with a similar proportion of patients reporting an adverse experience in ALT-711 and placebo groups, as reported in [86]. Another phase II clinical trial was initiated by Kitzman et al. to examine the effectiveness of ALT-711 in diastolic dysfunction. Patients were receiving 210 mg ALT-711 twice a day on an open-label, out-patient basis for at least 12 weeks. A reduction in left ventricular mass and left ventricular diastolic filling was observed, as mentioned in [86]. Recently, 23 patients, (mean age: 71 years), with stable diastolic heart failure, were enrolled in a 16-week open-label trial. Treatment with ALT-711 (410 mg, daily) in patients with diastolic heart failure resulted in a decrease in left ventricular mass and improvement in left ventricular diastolic filling and quality of life [87].

4.2. RAGE blockers: sRAGE, anti-RAGE antibodies and signaling inhibitors

Other approaches to prevent AGE-mediated damage include (A) trapping of circulating AGEs before their binding to AGE receptors, (B) inhibition of AGE interaction with its receptor and (C) inhibition of signal transduction mediated by AGE receptor activation [88].

The prototype “drug” for the trapping of AGE ligands is *soluble RAGE* (sRAGE) which is the truncated form of RAGE, constituted by the extracellular ligand-binding domain of the receptor. In db/db mice, treatment with murine sRAGE (50 mg/day, IP, for 19 weeks) decreased albuminuria, glomerulosclerosis and GBM thickening [89]. In STZ-diabetic and apolipoprotein-E-deficient mice, administration of sRAGE for 6 weeks inhibited expression of VCAM-1, tissue factor, TGF- β , fibronectin and type IV collagen in aorta [90]. Furthermore Bucciarelli et al. [91] demonstrated, in the same animal model, that sRAGE was able to suppress established atherosclerosis. Administration of sRAGE in db/db mice restored effective

wound healing and decreased levels of cytokines such as TNF- α , IL-6 and of metalloproteinase-2,-3 and -9 [92].

Recently, it has been found that human vascular cells express a novel splice variant coding for a soluble RAGE protein named *endogenous secretory RAGE* (esRAGE). It neutralizes AGE effects in endothelial cells and is present in human serum [93]. Interestingly, decreased plasma levels of esRAGE were found in patients with essential hypertension [94], rheumatoid arthritis [95] or renal insufficiency [96].

Another endogenous AGE trapping system is *lysozyme*. Lysozyme has been reported to accelerate renal AGE clearance, to inhibit AGE-promoted PDGF- β and type IV collagen expression in mesangial cells and to improve albuminuria in db/db mice [97].

Inhibition of AGE-RAGE interaction may be realized by *anti-RAGE antibodies*. Indeed, administration of an anti-murine RAGE antibody for 2 weeks in db/db mice resulted in a decrease in kidney weight, mesangial volume and urinary albumin excretion and normalization of creatinine clearance and GBM thickness [98]. Same results were observed in a type 1 diabetes model [99].

Cerivastatin, a hydroxy-methyl-glutaryl-CoA inhibitor and *cucurmin* can inhibit signal transduction mediated by AGE receptor activation. Addition of cerivastatin or cucurmin to cultured human endothelial cells resulted in complete inhibition of the AGE-induced increase in NF- κ B and activator protein-1 (AP-1) activity and VEGF mRNA upregulation [100].

4.3. Other therapeutic compounds which were found subsequently to possess

4.3.1. Anti-diabetic drugs: metformin and pioglitazone

In addition to the *indirect effect of all anti-diabetic agents on AGE formation by lowering blood glucose level*, some anti-diabetic agents possess also *direct AGE inhibitor activity*.

Metformin is a biguanide compound used in the management of type 2 diabetes which has structural similarities to aminoguanidine. In vitro studies indicated that metformin could react with reactive dicarbonyl species, mainly methylglyoxal or glyoxal, to form guanidine-dicarbonyl adducts [101,102].

In STZ-diabetic rats, metformin at high dosage (500–650 mg/kg daily for 10 weeks) could reduce AGE deposition in sciatic nerve and renal cortex, in a dose-dependent manner, with improvement of sciatic nerve conduction velocity [103].

In type 2 diabetic patients, administration of metformin, for 2 months at various dosages, resulted in decrease in methylglyoxal (but not in 3-deoxyglucosone) plasma levels, only with the highest dosage (> 1 g/day) [104].

Pioglitazone is a member of the family of thiazolidine-dione compounds, sensitizing peripheral tissues to insulin. Its structure is similar to that of the thiazolidine derivative OPB-9195. In vitro studies indicate that pioglitazone is also an inhibitor of glycation and AGE formation [105]. Its mechanism of action is yet to be determined. However, administration of pioglitazone to KK/Ta mice resulted in decrease in albuminuria, independently of systemic blood pressure or blood glucose level [106].

4.3.2. Angiotensin converting enzyme inhibitors (ACEIs), vasopeptidase inhibitors (VPIs) and angiotensin II receptor inhibitors (AIIRIs)

Ramipril, an ACEI, and valsartan, an AIIRI, have been found to prevent albuminuria and to attenuate glomerular ultrastructural changes in STZ-diabetic rats [107]. Miyata et al. [108] showed that these effects could be linked, at least in part, to an inhibition of AGE formation: *in vitro* olmesartan, an AIIRI, and ramipril, an ACEI, at the same molar concentration, inhibited formation of pentosidine and CML during incubation of non-uremic-diabetic, non-diabetic-uremic or diabetic-uremic plasmas. They were more efficient than AMG or PM. Unlike AMG or PM, they do not trap reactive dicarbonyl precursors of AGEs, but inhibit their production by *chelating transition metals* and blocking various oxidative steps including hydroxyl and carbon-centered radicals, mainly at the pre-Amadori and only to a lesser extent at the post-Amadori steps of AGE formation (Fig. 3). These effects are common to six tested AIIRIs: olmesartan, candesartan, irbesartan, losartan and valsartan. A similar but milder effect is observed with four ACEIs: temocaprilat, enalaprilat, captopril and perindoprilat.

These observations were confirmed *in vivo*. Ramipril (3 mg/l in drinking water for 12 weeks) *attenuated renal AGE accumulation* in STZ-diabetic rats. However RAGE overexpression and NF-KB activation were not affected by the treatment [109]. Similar reno-protective effects were observed with valsartan in STZ-diabetic rats [110] and olmesartan in AGE-treated rats [111]. In STZ-diabetic rats, ramipril (3 mg/kg in drinking water for 24 weeks), as AMG, completely prevented glomerular PKC activation and albuminuria, suggesting that inhibition of PKC activity could be linked to the reno-protective effect of the compound [112]. Interestingly, it was observed in SHR, that combination of perindoprilat (ACEI, 2 mg/l) and of AMG (1 g/l) offers superior reno-protection than that observed with each monotherapy [113].

ACE inhibition modulates expression of the splice variants of RAGE [114]. Incubation of bovine aortic endothelial cells in high glucose with ramipril showed *increase in sRAGE secretion*. In STZ-diabetic rats, ramipril (3 mg/l in drinking water for 24 weeks) reduced levels of skin collagen CML and pentosidine as well as circulating and renal AGEs. Upregulation of the renal genes of all three variants of RAGE, particularly the splice variant C (sRAGE), was observed in animals treated by ramipril. sRAGE protein levels were increased in renal cells showing AGE-binding ability. Plasma sRAGE level was restored by ACEI. Similarly, in *type 1 diabetic patients* treated with the ACEI perindopril for 24 months, increase in plasma sRAGE was observed as compared with placebo-treated patients. Thus, it has been proposed that ACE inhibition may reduce the accumulation of AGEs in diabetes partly by increasing production and secretion of sRAGE. However, ACEI and AIIRI could differ in their mechanism of action since, in the KK/Ta mice, candesartan (AIIRI, 4 mg/kg daily from 6 to 28 weeks of age) reduced renal AGE accumulation, albuminuria, mRNA and protein expression of p47phox, iNOS and RAGE expression [115].

Recently, it has been found that VPIs combining ACE and neutral peptidase inhibition could be potent AGE inhibitor. AVE7688, a VPI, (but not the ACEI ramipril) attenuated renal accumulation of 3-deoxyglucosone-imidazolone, pentosidine and CML, in Zucker diabetic fatty rats. The doses of AVE 7688 and ramipril have been chosen to present the same inhibitory activity towards ACE. During glycation reactions *in vitro*, AVE 7688 demonstrated potent chelating activity and inhibited metal-catalyzed formation of pentosidine and CML [116]. In diabetic apolipoprotein-E-efficient mice, omapatrilat, another VPI, reduced atherosclerosis, renal structural injury and albuminuria. Omapatrilat conferred superior reno-protection than the ACEI quinalapril [117].

4.3.3. Aldose reductase inhibitors (ARI)

Under hyperglycemic conditions, excess glucose is metabolized into sorbitol by the AR pathway. This process may be pursued by fructose formation leading to 3-deoxyglucose and methylglyoxal (Fig. 4), which are known to accelerate formation of AGEs. Moreover, it has been found that AGEs can upregulate gene expression of AR in smooth muscle cells leading to increased activity in cultured smooth muscle cells as well as in incubated aortic strips [118].

Sorbinil, an ARI decreased AGE-related fluorescence in skin collagen of diabetic rats [119]. However, Cohen et al. [120] showed that treatment with sorbinil for 30 days *in STZ-diabetic rats* lowered glomerular fructose concentration, but did not influence collagen fluorescence in GBM. Ponalrestat, another ARI, retarded fluorescence in aorta but not in glomeruli and renal tubules, of *STZ-diabetic rats* [121]. In diabetic dogs, administration of sorbinil for 5 years prevented sorbitol accumulation in erythrocytes and defective nerve conduction, but had no beneficial effects on renal structure or albuminuria [122,123].

In *type 2 diabetic patients* treatment with epalrestat (150 mg daily) for 2 months lowered erythrocyte CML level, without changes in glycemia [124]. This effect might be linked to the metal-chelating and antioxidant properties of AR inhibitors reported in erythrocytes of diabetic rats [125]. In another study, plasma CML concentration did not change in *type 2 diabetic patients* after the administration of epalrestat for 3 months except in the patients whose CML concentration before treatment was higher than 3 mU/ml [126].

4.3.4. Anti-inflammatory drugs: diclophenac and salicylates

Studies in STZ-diabetic rat [127] or in diabetic patients [128] have shown that anti-inflammatory drugs such as aspirin, salicylates or ibuprofen can protect against diabetic cataract. This led to the suggestion that aspirin and others anti-inflammatory drugs might interfere with AGE formation. In collagen fibers incubated with glucose, *aspirin* decreased thermal rupture time [129]. In the same type of model, aspirin was found to inhibit pentosidine formation [130,131]. Aspirin and salicylates may act as free radical scavengers and/or metal ion chelators [132].

In STZ-diabetic rats, aspirin or sodium salicylate treatment for 4 weeks (240 mg/kg, daily) was able to prevent the rise in thermal rupture time without affecting glycation [129]. In diabetic dogs, aspirin (20 mg/kg daily for 5 years) inhibited development of retinal hemorrhages and acellular capillaries over the 5 years period, but did not affect albuminuria or nerve conduction velocity deficit. No effect in accumulation of pentosidine or immuno-reactive AGEs in aorta or tail tendon collagen was observed [33]. In type 2 diabetic patients, aspirin treatment (100 mg daily for 1 year) decreased skin pentosidine levels [133].

In vitro studies have shown that diclofenac can block at least one of the major glycation sites of human serum albumin [134]. In diabetic db/db mice, administration of diclofenac (3 mg/kg twice daily) normalized plasma concentration of glycosylated albumin within days after initiation of treatment and maintained glycosylated albumin within the normal range throughout the study (12 weeks); it also reduced the overexpression of mRNA encoding for type IV collagen in renal cortex [135].

4.3.5. Tenilsetam

Tenilsetam or 3-(2-thienyl)-2-piperazinone is an anti-dementia drug used for the treatment of Alzheimer's disease. During incubation of lysozyme or collagen with high glucose, tenilsetam inhibited lysozyme polymerization due to advanced glycation and prevented reduction of collagen digestibility, in a dose-dependent manner. In STZ-diabetic rats, tenilsetam (50 mg/kg daily for 16 weeks) inhibited AGE-fluorescence in renal cortex and aorta. The mechanism of action remains to be elucidated [136].

4.3.6. Dietary antioxidants

Antioxidants may protect against glycoxidation, glucose autooxidation and lipoxidation.

Vitamin E (800 mg/day) was reported to reduce AGE accumulation in arterial walls of diabetic patients [137]. However it failed to efficiently prevent diabetic complications [138].

Vitamin C is antioxidant, however dehydroascorbic acid is a potent glycosylating agent, particularly in the aging human lens [139].

Flavonoids are present in plant-derived foods. They show important antioxidant and AGE inhibitor properties, according to their structure, in vitro [140] and in vivo: they decrease skin collagen-linked fluorescence in diabetic rats [141]; besides they decrease albuminuria and restore albuminemia [142]. These dietary antioxidants of low toxicity could be promising for the treatment of diabetic complications, although their therapeutic potential in humans remains to be investigated.

5. Conclusion

Strict glycemic control is the first therapy for reducing AGE formation in diabetes. However for a similar glycemic control, patients appear to be more or less susceptible to diabetic complications, suggesting a genetic control of oxidant and/or carbonyl stress. Skin AGEs appear to be powerful predictors of

the risk of developing diabetic complications even after adjustment for mean HbA_{1c} [143]. This underlines the interest of AGE inhibitors or breakers. Despite encouraging results obtained in vitro and in animal studies, most of the clinical trials have been more or less disappointing, in part because of side effects; the long-term therapeutic interest of the most recently developed AGE inhibitors or breakers remains to be demonstrated.

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