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Minireview

Angiotensin II receptor blockers and angiotensin converting enzyme inhibitors: implication of radical scavenging and transition metal chelation in inhibition of advanced glycation end product formation

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The Maillard reaction irreversibly modifies proteins over time to form advanced glycation end products (AGEs).[1] AGEs are thought to contribute to the development of atherosclerosis and of diabetic and uremic complications [1–5]. Inhibition of AGE formation has thus become a therapeutic goal. In this review, we analyze two categories of compounds shown to decrease AGE levels, i.e., OPB-9195 (a hydrazine-derivative) and angiotensin II receptor blockers (ARB)/angiotensin converting enzyme inhibitors (ACEI). We elucidated their mechanisms of action and propose a comprehensive mechanism for the action of AGE lowering drugs.

OPB-9195, a hydrazine-derivative

Aminoguanidine, the first AGE inhibitor discovered in 1986 [6], was followed by a more effective compound, (±)-2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide (OPB-9195) [7]. Both are hydrazine-derivatives. In vitro, OPB-9195 inhibits the formation of two AGE moieties, pentosidine [8] and N^ε-carboxymethyllysine (CML) [9], from a variety of individual precursors including ribose, glucose, and ascorbate, as well as that of two advanced lipoxidation end product (ALE) moieties, malondialdehyde-lysine and 4-hydroxynonenal-protein adduct, from arachidonate [10]. It also inhibits pentosi-

dine generation from diabetic and uremic plasma [11] or conventional glucose based peritoneal dialysis fluid fortified with bovine serum albumin [10].

The chemical and biologic mechanisms of the inhibitory effect of hydrazine derivatives on AGE formation are still under investigation. The hydrazine nitrogen atom of OPB-9195 reacts with carbonyl precursors for AGEs directly, or upon hydrolysis, via the free base to form hydrazone indirectly [10]. This mechanism is similar to that proposed for aminoguanidine (aminoguanidine has the additional binding site for carbonyl precursors, i.e., guanidine group) [10]. Structurally, OPB-9195 is expected to be more effective than aminoguanidine, because the latter's hydrazine nitrogen atom has a decreased nucleophilicity due to the proximity of the guanidinium cation. Indeed, the in vitro formation of pentosidine in uremic plasma [11] and of protein carbonyls [12] is more effectively inhibited by OPB-9195 than by aminoguanidine.

OPB-9195 corrects several biological effects associated with AGE formation. In murine thymocyte and fibroblast cultures, it inhibits the phosphorylation of tyrosine residues of a number of intracellular proteins induced by cell surface Schiff base formation [13]. In the experimental diabetic animal, such as the Otsuka–Long–Evans–Tokushima–Fatty (OLETF) rat, it reduces urinary albumin excretion and improves glomerular morphology [7]. Given to rats, after balloon injury of their carotid arteries, it effectively reduces neointima proliferation in arterial walls [14].

Unfortunately, clinical benefits of this compound given to diabetic patients have been hampered by its side effects, related to the characteristic trapping of pyridoxal with an attendant vitamin B6 deficiency [15].

Angiotensin II receptor blockers and angiotensin converting enzyme inhibitors

We searched for other drugs whose tolerance had been demonstrated in clinical conditions and which might inhibit AGE formation. Highly sensitive and specific chemical methodologies for AGE determination [16,17] were utilized rather than more disputable physicochemical (fluorescence and cross-linkage on electrophoresis) immunological assays. Unexpectedly, angiotensin II receptor blockers (ARB) and angiotensin converting enzyme inhibitors (ACEI) were found to lower AGE formation [15].

In vitro production of two AGEs, pentosidine and CML, is inhibited in a dose-dependent manner by two anti-hypertensive agents, olmesartan (ARB) and temocaprilat (ACEI), but not by nifedipine, an anti-hypertensive calcium blocker. Inhibition is demonstrated in incubation media containing either arabinose, non-uremic diabetic plasma, non-diabetic uremic plasma, or diabetic uremic plasma as the sources for AGEs. It is more striking for olmesartan and temocaprilat than for aminoguanidine at the tested concentrations [15].

The effect on AGE formation is common to 6 tested ARB, e.g., olmesartan, candesartan, irbesartan, losartan, termisartan, and valsartan, including their common core structure, 5-(4′ methylbiphenyl-2-yl)-1H-tetrazol. A similar but milder effect on AGE formation is observed with 4 ACEI tested at similar concentrations, e.g., temocaprilat, enalaprilat, captopril, and perindprilat. No common core structure was identified in the tested ACEI.

Mechanisms of AGE inhibition by ARB/ACEI

RCO trapping

Reactive carbonyl and dicarbonyl compounds (RCOs) are critical precursors of AGEs [18–21]. Trapping of RCOs such as glyoxal and methylglyoxal is involved in inhibition of AGE formation by several compounds, e.g., aminoguanidine, pyridoxamine, and OPB-9195. In contrast, olmesartan and temocaprilat do not react with RCOs, suggesting therefore that their AGE inhibitory effect is independent of RCO trapping [17].

Of note, olmesartan and temocaprilat do not react with pyridoxal, in contrast with hydrazine derivatives, aminoguanidine and OPB-9195 [17].

Effect on oxidative metabolism and dicarbonyl production

The production of RCO precursors for AGEs is in part linked with the oxidative metabolism [22]. In vitro, temocaprilat and, especially, olmesartan decrease in a dose-dependent manner the level of carbon-centered radicals, unlike aminoguanidine [15]. Both drugs also reduce the level of hydroxyl radicals, a characteristic shared to a minor extent by aminoguanidine.

Interestingly, the inhibition of the oxidative metabolism by olmesartan is matched by a decreased production of glyoxal and methylglyoxal [15].

Transition metal chelation

The formation of AGEs, CML, and pentosidine, is closely related to the presence of transition metal ions [23,24]. The chelating activity of the AGE lowering compounds for transition metal ions was therefore assessed. The drug concentration required for a 50% inhibition of the rate of copper-catalyzed autoxidation of ascorbic acid in phosphate buffer was measured. Among the tested compounds, 5-(4′-methylbiphenyl-2-yl)-1H-tetrazol (a core structure of ARB) and olmesartan as well as OPB-9195 chelated copper and inhibited the autoxidation of ascorbic acid in a concentration-dependent manner, to a much greater extent than aminoguanidine [15]. Temocaprilat has an intermediary effect.

The mechanism of the AGE lowering effect of ARB/ACEI thus differs markedly from those of aminoguanidine and OPB-9195. Neither ARB nor ACEI entraps and lowers in vitro the concentration of RCOs whereas aminoguanidine and OPB-9195 reduce RCO levels at equimolar concentrations. Alternatively, ARB and ACEI decrease RCO production by interfering at least with the two oxidative steps, i.e., the formation of carbon-centered radicals and hydroxyl radicals, a characteristic shared only to a minor extent by aminoguanidine. ARB and ACEIs further chelate transition metals implicated in the acceleration of AGE formation.

The in vitro effects of ARB and ACEI on AGE formation have been obtained at drug concentrations one order of magnitude above those achieved under clinical circumstances. The dose–effect relationship between lower concentrations of olmesartan and temocaprilat, from 0.01 to 800 μM, and pentosidine generation was therefore delineated [15]. The dose responses proved to be curvilinear for both drugs, demonstrating thus an effect at far lower concentrations. As previously reported by the group of Baynes [25] and described in the following section, the in vivo inhibition of AGEs is indeed achieved at these far lower concentrations. Such discrepancies between in vivo and in vitro data are not unusual in pharmacology and may be related to a much longer drug exposure in vivo than in in vitro assays (see Fig. 1).

Association of AGE inhibition and renoprotection in the experimental diabetic model

A spontaneously hypertensive/NIH-corpulent rat [SHR/NDmc-cp (fat/fat)] strain [26] has been used to assess the effect of ARB on in vivo AGE formation [27].

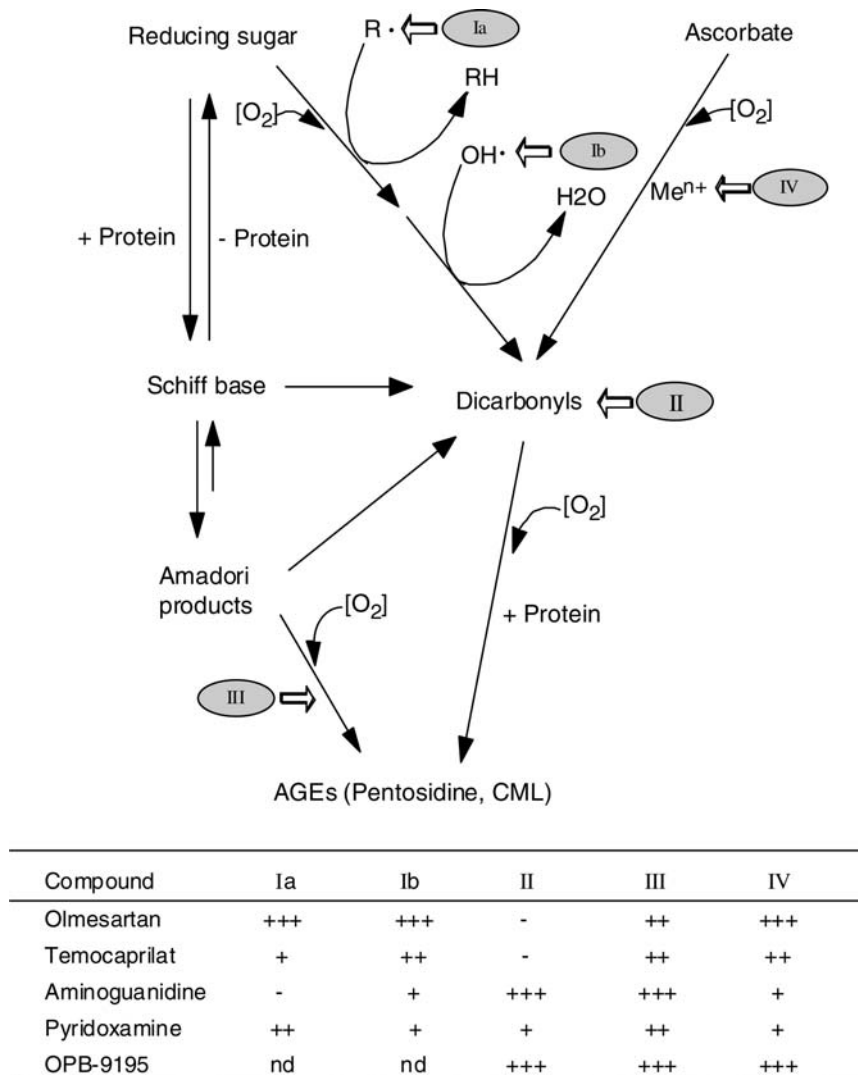| Compound	Ia	Ib	II	III	IV
Olmesartan	+++	+++	-	++	+++
Temocaprilat	+	++	-	++	++
Aminoguanidine	-	+	+++	+++	+
Pyridoxamine	++	+	+	++	+
OPB-9195	nd	nd	+++	+++	+++

Fig. 1. Mechanisms of drug-induced AGE inhibition. Reducing sugars are autoxidized to their carbonyls or dicarbonyls, and reduced molecular oxygen to form superoxide/carbon-centered/hydroxyl radical. These radicals further promote the chain reaction of sugar oxidation. Some compounds trap carbon-centered radicals (Ia) and hydroxyl radicals (Ib) to inhibit whole sugar-autoxidation process and, consequently, suppress carbonyl/dicarbonyl and the attendant AGE formation. Some compounds trap carbonyls by themselves (II) or inhibit the post-Amadori AGE formation (III). In addition, these compounds chelate transition metal ions, potent catalysts of the AGE formation (IV). ND, not determined due to the low solubility of OPB-9195 in the reaction mixture. Reprinted with permission (from [15]).

This model, contrary to other models such as the streptozotocin-induced diabetic rats, is relevant to human type II diabetes, as it exhibits obesity, early hypertension, hyperglycemia with hyperinsulinemia, proteinuria, and glomerular lesions resembling human diabetic glomerulosclerosis.

Olmesartan given to SHR/NDmc-cp (fat/fat) did not modify body weight or plasma levels of glucose and insulin, but decreased plasma levels of total cholesterol and phospholipid. It reduced in a dose-dependent fashion systolic blood pressure and diabetic nephropathy as evidenced by a decrease in proteinuria and in pathologic evidence of glomerulosclerosis.

The renal content of pentosidine was significantly higher in the SHR/NDmc-cp (fat/fat) group given only vehicle than in both spontaneously hypertensive rats (SHR) and control Wister–Kyoto rats, despite similar genetic background and the fact that systemic blood pressure was much higher in SHR than in SHR/NDmc-cp (fat/fat). It is thus unlikely that the genetic background or hypertension itself enhances the formation of AGEs. Administration of two different doses of olmesartan (1 and 5 mg/kg/body weight/day) to SHR/NDmc-cp (fat/fat) reduced the renal pentosidine content in a dose-dependent way. Of note, the renal pentosidine content proved significantly correlated with proteinuria, supporting the existence of a link between AGE formation and renal damage.

The drug dosage used is clinically relevant. The plasma levels achieved in rats are close to the pharma-

cologically effective levels in healthy human volunteers: olmesartan (10 mg/kg body weight/day) yields peak plasma levels of 1.3 μg/ml in rats to be compared with 0.4–0.5 μg/ml peak plasma levels for olmesartan given to human volunteers [27].

Despite their severe hypertension, non-diabetic SHR rats exhibit only a slight increase of proteinuria and glomerular damage. This finding points to the critical role played by sustained hyperglycemia with high insulin levels and/or obesity related hypercholesterolemia in the genesis of the marked renal lesions observed in SHR/NDmc-cp (fat/fat). The attendant advanced glycation of proteins, identified here by a high renal pentosidine content, might be a key element of the enhanced susceptibility of the kidney. It is indeed associated with a number of cellular events, summarized previously [28], whose downregulation by AGE inhibitors may contribute to the protection of kidney. Alternatively or concurrently, lowering of pentosidine might reflect a decreased oxidative stress with a double consequence: reduced production of RCO precursors of pentosidine and reduced direct oxidative damage to proteins. Of course, in SHR/NDmc-cp, hypertension together with metabolic derangement of diabetes, e.g., hyperglycemia and oxidative stress, are at the root of both pentosidine formation and proteinuria.

Further evidence in favor of our interpretation has been recently reported in rats with streptozotocin induced diabetes. The group of Baynes [25] demonstrated that an AGE inhibitor, pyridoxamine [29], reduced AGE formation and contributed to the renoprotection. Subsequently, Forbes et al. [30] reported that ramipril (ACEI) and aminoguanidine reduced to the same extent AGE accumulation in the kidney as measured by immunohistochemistry and fluorospectrometry. Finally, Wilkinson-Berka et al. [31] observed that aminoguanidine as well as another AGE inhibitor, ALT-946, decreased AGE immunolabeling and provided renal protection.

The beneficial effects of olmesartan on diabetic renal damage are especially noteworthy as they are manifest despite sustained hyperglycemia. A similar observation has been made in experimental diabetic rats given AGE inhibitors without anti-hypertensive effect, e.g., aminoguanidine, ATL-946, OPB-9195, and pyridoxamine [25,29–32]. These data thus suggest a direct role of AGE formation in the development of diabetic renal complications.

These considerations provide a framework to understand the additional benefits, beyond those derived from blood pressure lowering, described in clinical studies of ARB in type II diabetics [33–35]. We suggest that significant renoprotection is derived not only from blood pressure lowering and angiotensin inhibition, but also from reduced AGE formation probably associated with a decreased oxidative stress. This hypothesis fits with the renoprotection afforded by AGE inhibitors in diabetic rats without hypertension [29–32] or by ACEI and ARB in normotensive human diabetics [36].

Weinberg et al. [37] have administered a supramaximal dose (up to 96 mg, far beyond the approved dose for this drug) of candesartan (ARB) to subjects with heavy proteinuria. Their preliminary data suggest that these high doses further reduce proteinuria without concomitant additional anti-hypertensive effect, despite complete angiotensin receptor blockade and an unchanged blood pressure. The chemical effect, rather than the biological action of this drug on angiotensin, seems instrumental in modifying proteinuria. The dose-dependent anti-oxidant activity (radical scavenger, transition metal chelation, etc.) of ARB could be the key to renoprotection.

It has been claimed that the additional benefit of ARB derives from its interference with the renin–angiotensin system. To evaluate this possibility, the efficacy of olmesartan was compared with that of hydralazine, another anti-hypertensive agent acting independently of the renin–angiotensin system [27]. Hydralazine reduces in vitro pentosidine and CML production by two mechanisms: first it traps RCOs (the same mechanism as aminoguanidine and OPB-9195) and second it scavenges reactive oxygen species and chelates transition metals (the same mechanism as ACEI and ARB) [27]. In vivo also, hydralazine (5 mg/kg/day) given to SHR/NDmc-cp rats reduces significantly the renal pentosidine content. It lowers and modifies albuminuria and glomerular damage to the same extent as olmesartan (low dosage, 1 mg/kg/day). This finding raises the possibility that the added benefits of ARB in clinical trials are not necessarily due to their ability to interfere with the renin–angiotensin system.

Still, more data are needed to confirm the unexpected AGE lowering effect of ARB and some ACEI, and to ascertain how these effects may contribute to demonstrated clinical and experimental benefits of these drugs.

Conclusion

Our approach provides a comprehensive outline of the mechanisms involved in the prevention of AGE formation. It further shows that available compounds may simultaneously act at different levels. OPB-9195 and hydralazine trap RCOs, chelate transition metals, and scavenge mildly carbon-centered and hydroxyl radicals. In contrast, both ARB and ACEI have no RCO trapping effect but strongly chelate transition metals and scavenge markedly the reactive oxygen species.

The discovery that ARB and ACEI reduce AGE formation raises prospects for new therapeutic interventions. Both families of compounds have been used

for many years and are well tolerated unlike the first AGE lowering agents, aminoguanidine and OPB-9195, which trap not only noxious RCOs but also pyridoxal.

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