Clinical Studies of Advanced Glycation End Product Inhibitors and Diabetic Kidney Disease

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Current treatment of the nephropathy complication of diabetes mellitus is suboptimal in halting the progression of the complex disease. Among the irreversible effects of sustained hyperglycemia is the heightened formation of advanced glycation end products (AGEs). The role of AGEs in diabetic nephropathy has been established by years of basic research. This article reports progression through human studies of the few AGE inhibitors that have reached clinical development, including pimagedine, pyridoxamine, and alagebrium.

Introduction

The projected worldwide increase in diabetes mellitus in the coming decades will create a compelling need for treatments of diabetic nephropathy. This common microvascular complication of type 1 and type 2 diabetes results in chronic kidney disease and end-stage renal disease, requiring dialysis and transplantation. Diabetic nephropathy has become the major cause of kidney failure in the western world and is projected to become the most frequent cause in the African continent and in developing countries in the future [1]. Using a cost-of-illness model, the total annual medical costs for managing type 1 and type 2 diabetic nephropathy in the United States reached nearly $17 billion early in this century [2].

With few current therapies proven to prevent or slow the progression of diabetic nephropathy [3–6], there is an ongoing search for new interventions of this complex metabolic disease. Among the irreversible effects of chronic hyperglycemia is the accelerated formation of advanced glycation end products (AGEs), created by a reaction between sugars and free amino acid groups on proteins, lipids, and nucleic acids. Circulating AGEs and tissue AGE deposits are characteristic of diabetic complications [7]. The role of AGEs in diabetic nephropathy has been developed over more than 15 years of AGE biochemistry (Fig. 1) [8•].

However, the increasingly complex and diverse AGE biochemistry continues to create new challenges for AGE inhibitory therapies [9]. AGEs are versatile structures with protean potential to create toxic biological effects [10]. More AGEs have been identified in recent years so that the role of AGEs—including AGE biochemical mechanisms, their range of chemical, clinical, and tissue biologic effects, and the molecular targets and effects of therapeutic agents—have become increasingly complex [11]. Because no AGE inhibitors have entered clinical practice [12], this article emphasizes the current state of AGE therapeutic agents.

AGE Biochemistry

Advanced glycation end products are permanent biochemically active glucose structures formed by nonenzymatic post-translational reactions [13]. AGE pathobiochemistry has been intensively studied in recent years. Their formation occurs through several key steps (Fig. 2). In the core reaction, reducing sugars react nonenzymatically with amino groups to form Schiff bases, followed by rearrangement to reversible Amadori products, relatively stable ketoamines. Amadori products are the predominant form of circulating glycated proteins in diabetic patients [10]. These glycated proteins then slowly undergo progressive dehydration, cyclization, oxidation, and rearrangement to form AGEs. More broadly, multiple sources and mechanisms of AGEs exist in vivo [14], and AGE formation involves a cascade of sequential and parallel reactions, in some cases poorly understood.

The early stages have in some cases been well characterized and identified for several proteins [15]. For example, AGEs arise from reactions of intracellular dicarbonyls such as glyoxal, methylglyoxal, 3-deoxyglucosone, and glucosone. When oxidation accompanies glycation, the products formed are glycoxidation products. The glycoxidative degradation of protein-carbohydrate adducts to AGEs is a complex pathway requiring oxygen and catalyzed by traces of copper and iron. AGEs can also form on the amino groups of lipids (advanced lipoxidation end products [ALEs]), DNA, and through a number of other pathways. Some AGE compounds may be derived from either carbohydrates or lipids.

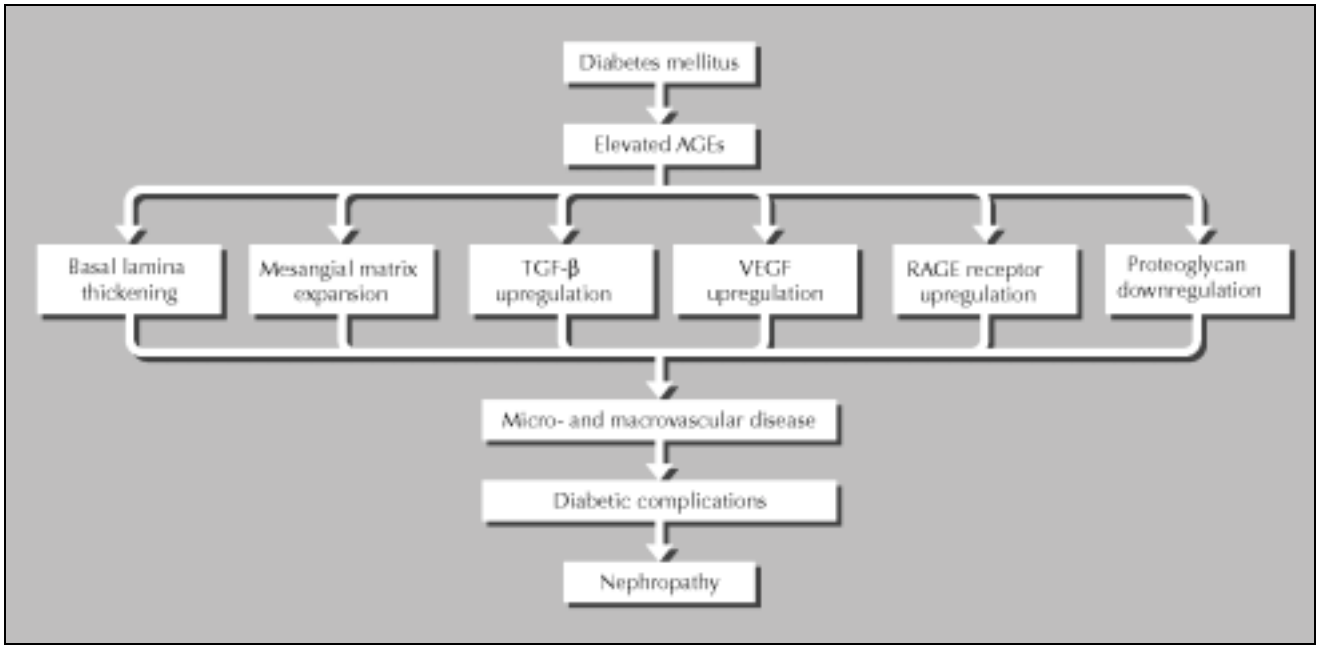

Figure 1. Role of advanced glycation end products (AGEs) in diabetic nephropathy. RAGE—receptor for advanced glycation end product; TGF-β—transforming growth factor-β; VEGF—vascular endothelial growth factor.

Many AGEs proceed to abnormal cross-link formation in diabetic patients, a stage of the AGE reaction that damages proteins and other macromolecules by altering their molecular configuration. Other AGEs are capable of triggering diverse injury responses after being taken up by specific cellular receptors.

AGEs and the Kidney

A significant body of evidence now implicates early, as well as advanced, glycation products in the pathogenesis of advanced diabetic nephropathy [16]. Normal rats exposed to Amadori products in infused plasma develop glomerular hyperfiltration, an early feature of diabetic nephropathy [17]. The AGE content of the kidney is increased when normal rats are infused with AGEs over several months. Glycated proteins injected into normal mice produce glomerular basement membrane thickening and glomerular lesions suggestive of diabetic glomerulopathy [18]. In diabetic animal models, AGEs accumulate in expanded mesangial matrix and nodular glomerular lesions. A growing list of AGE compounds has been determined in the diabetic kidney [19], either as a result of mesangial trapping of circulating AGEs, through tubular reabsorption of AGE peptides, or by AGEs formed intrinsically in the diabetic kidney. AGE receptors are known to exist in the kidneys [20].

Both of the dominant histologic features of diabetic nephropathy, mesangial expansion and basement thickening, could result from changes in mesangial matrix composition. Accumulation of extracellular matrix proteins in the glomerular mesangium is characteristic of diabetic nephropathy. Normal collagen and matrix-cell interactions may be damaged by AGEs [21•]. Cross-linking of basement membrane proteins may increase permeability and lead to glomerular passage of proteins (*eg*, microalbuminuria, proteinuria). AGEs may also induce formation of profibrotic mesangial calls and release of proinflammatory cytokines and adhesion molecules.

Pharmacologic inhibitors of AGE formation or ultimate cross-linking have been pursued in experimental models of diabetic nephropathy for several years. No common structure exists in compounds being evaluated as AGE inhibitors. Mechanisms of AGE inhibition by therapeutic drugs include trapping of carbonyls, carbon-centered radicals, or hydroxyl radicals, chelating transition metal ions, inhibiting post-Amadori AGE formation, or breaking cross-links. Only a few evaluated inhibitors have reached clinical development (Table 1).

Pimagedine

Pimagedine, the prototype therapeutic agent for prevention of AGE formation, entered clinical development several years ago [22]. In animal models, the drug inhibited the formation of AGEs [23] and was effective in reducing the severity of the structural and functional alterations of diabetic nephropathy [24•]. Pimagedine is a competitive inhibitor of the AGE pathway that reacts with dicarbonyl compounds as well as other biologic molecules [25].

The pivotal phase 3 pimagedine trial, which reached publication in 2004 [26], enrolled 690 patients with type 1 diabetic nephropathy in a randomized placebo trial with an average drug treatment time of 2.5 years. Pimagedine (20%) produced no significant difference from placebo (26%) in the primary outcome, doubling of serum creatinine. Pimagedine did produce some reduction in urinary protein

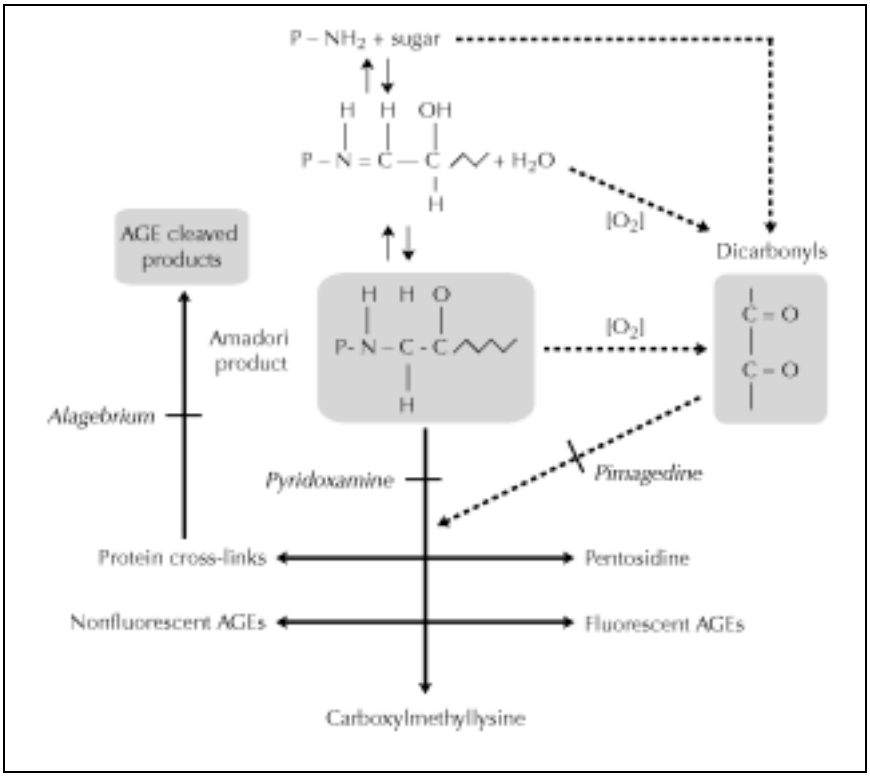

Figure 2. Simplified advanced glycation end product (AGE) formation pathways and inhibitory actions of candidate therapeutic AGE inhibitors.

excretion and lipid levels, and appeared to limit progression of diabetic retinopathy. The protocol was not powered to take into account the beneficial effects of angiotensin-converting enzyme inhibitors (ACEIs)/angiotensin receptor blockers (ARBs), which the majority of enrolled patients in both groups received. Furthermore, the study drug pimagedine was associated with a high rate of complications and discontinuations. Relevant pimagedine-related adverse events included transient flu-like syndrome, anemia, and induction of antinuclear and antineutrophil cytoplasmic antibodies (ANCAs). Three patients on pimagedine in the higher dose arm developed crescentic glomerulonephritis, associated with very high ANCA levels. As a result, the clinical development of pimagedine is now suspended.

Pyridoxamine

The AGE inhibitor pyridoxamine has entered clinical development as an effective inhibitor of the formation of AGEs that arise from Amadori products. Pyridoxamine is a derivative of vitamin B_6. It inhibits both the formation of AGEs and the development of complications in animal models of diabetes. Pyridoxamine inhibits AGE and ALE formation in vitro, and inhibits the chemical modification of proteins in both hyperglycemic and hyperlipidemic animal models [27]. Unlike other known AGE inhibitors, pyridoxamine is known to inhibit the degradation of protein-Amadori products to protein-AGEs (*ie,* it is a post-Amadori inhibitor).

Two predominant mechanisms for decreasing the formation of AGEs have been proposed: carbonyl trapping [28], and scavenging of metal ions in vivo. It has been proposed that the drug blocks Amadori-to-AGE conversion by interfering with the catalytic role of redox metal ions required for this glycoxidative reaction. The original discoverers of pyridoxamine have recently proposed, based on in vitro studies, that the compound primarily blocks conversion of Amadori intermediates to AGE-carboxymethyllysine. The mechanism of inhibition involved interference with the catalytic role of redox metal ions required in vitro for the glycoxidative reaction. The inhibition of pyridoxamine by binding to required redox metal ions and blocking their catalytic role occurred in vitro under concentrations of glucose found in the diabetic state [28].

Because carboxymethyllysine is not the sole product of degradation of Amadori intermediates, additional mechanisms may apply in vivo. One alternative is the chemical trapping of low molecular weight reactive carbonyl products of glucose and lipid degradation. It was recently proposed that pyridoxamine is a potent scavenger of 1,4-dicarbonyl compounds, and that the structural phenolic group of pyridoxamine was essential to its reactivity for dicarbonyl compounds [29]. In vivo, the relative contributions of these mechanisms may be influenced by local concentrations of redox metal ions and of reactive carbonyls [30].

Pyridoxamine is also known to be an effective inhibitor of ALE in vitro [31]. In analogy to the above mechanisms, trapping of intermediates of AGE formation has also been proposed for pyridoxamine inhibition of lipid peroxidation reactions [26]. Adducts of linoleate and arachidonate were formed after in vitro incubation and also in the urine of pyridoxamine-treated animals in one recent study [26].

Table 1. Pharmacologic AGE inhibitors and their renal effects in experimental and human studies

Class	Compound	In vitro	Animal studies	Clinical trials
AGE inhibitor	Pimagedine	Reacts with glucose-derived intermediates	↑ Tissue AGE levels, ↓ AGE binding, prevents ↑ fluorescence in glomeruli and tubules, ↓ mesangial matrix expansion, ± ↓ glomerular basement membrane thickening, ↓ proteinuria, ↑ survival in azotemic model	Mixed results in phase 3. Primary renal end point did not reach statistical significance. Slight reduction in proteinuria. Poor safety profile
AGE inhibitor	Pyridoxamine	Inhibits AGE formation from Amadori intermediates	Protects kidney from AGE-modified albumin injection, ↓ glomerular volume, ↓ albuminuria	Phase 2 safety and efficacy trials completed. Good safety profile. Preliminary efficacy results encouraging
Cross-link breaker	Alagebrium	Breaks AGE collagen cross-links	↓ Diabetic rat tail collagen cross-links, ↓ renal tissue AGEs, restores vascular compliance of large vessels	Phase 2 cardiovascular trials completed

AGE—advanced glycation end product.

Several preclinical studies have now demonstrated that oral pyridoxamine is protective in a preventative model of type 1 (streptozocin rat model) [32] and type 2 (db/db mouse model) diabetic nephropathy. As an inhibitor of ALEs during hyperlipidemia [33], pyridoxamine lowers both plasma cholesterol and triglycerides, and prevents the chemical modification and cross-linking of renal and vascular collagen in diabetic and Zucker obese rats. The compound protects against the pathologic changes of early diabetic nephropathy induced by injections of AGE-modified rat serum albumin.

In 2003, pyridoxamine advanced into clinical studies of diabetic nephropathy. A phase 2 study in the United States was undertaken to determine the safety and tolerability and provide preliminary observations on its efficacy, in patients with type 1 and type 2 nephropathy with baseline serum creatinine ≤ 2.0 mg/dL and overt proteinuria [34]. Baseline demographic and laboratory characteristics were comparable, and most patients were already receiving ACEI/ARB therapy. The drug was well tolerated, and the percentage of patients who experienced treatment-related adverse events was low and comparable in both treatment and placebo groups. Preliminary efficacy analysis indicated that a rise in serum creatinine of less than 0.5 mg/dL was more common in patients on placebo (22%) than on pyridoxamine (12%). Post hoc analysis indicated that statistically significant reductions in the rise of serum creatinine and urinary albumin excretion were seen in the treatment group. In another trial, preliminary evidence of efficacy for pyridoxamine on kidney function was again demonstrated [35], and the treatment group exhibited a decrease in urinary transforming growth factor-β, the profibrotic cytokine [36], which has been associated with glomerulosclerosis [37]. A longer phase 3 trial properly designed to evaluate drug efficacy in diabetic nephropathy is planned.

Alagebrium

Protein cross-linking is mediated by AGEs and involves glycated molecules bound together. Collagen cross-linking, for example, occurs at an accelerated rate in diabetic patients. Cross-link breakers may have therapeutic potential to reverse already formed cross-links, and correct glycation damage, even in the presence of inadequate glucose control. Specific mechanisms of cross-link activity may involve breaking of bonds between two carbonyl groups in compounds responsible for cross-link reactions [14]. However, activity in vitro to cleave model cross-links experimentally may not apply to diverse AGE cross-links in vivo.

Advanced glycation end product breakers have been shown in vitro and in vivo to cleave cross-links in diabetic animal models. Alagebrium (formerly ALT-711) is a derivative of phenacylthiazolium bromide, the prototype cross-link breaker, and is chemically related to vitamin B_1 [38]. In a rat model of diabetes, cross-link breaking activity of alagebrium was measured as a reduction in immunoglobulin G covalently found to erythrocytes. Immunoglobulin G is covalently bound to erythrocytes in AGE cross-links, which are increased in diabetic subjects. A known clinical target of AGE cross-linking in vivo is collagen, a matrix protein more tightly cross-linked in the presence of diabetes. Increased collagen cross-linking may contribute to the vessel wall and myocardial stiffness associated with diabetes. Experimental studies indicate that alagebrium increases large vessel compliance [39] and restores left ventricular collagen solubility [40]. Additional animal studies have confirmed cardiovascular benefit, and suggest amelioration of diabetic nephrosclerosis [41•]. Current clinical trials, including one showing improved arterial compliance in humans with vascular stiffness [42], are evaluating the cardiovascular benefit of alagebrium. Little is known about the renoprotective potential of AGE cross-link breakers in human diabetic nephropathy [43].

Glycated Albumin

Serum albumin is modified by Amadori glucose adducts, and Amadori-modified albumin is the predominant glycated protein in the circulation. Over the past few years, increasing evidence, principally from Cohen *et al.* [44], has suggested that glycated albumin contributes to diabetic kidney disease. In cultured renal cells, Amadori-glycated albumin produces pathophysiologic effects that duplicate exposure to high glucose levels, including stimulation of protein kinase C, increased transforming growth factor-β activity, and enhanced production of extracellular matrix proteins [45]. Glycated albumin modulates signal transduction and induces these alterations in renal glomerular cells, which are felt to contribute to the development of diabetic nephropathy [44]. Additional studies suggest that Amadori-configurated glycated albumin may contribute to early diabetic hyperfiltration by enhancing nitric oxide synthase activity [46]. Glycated albumin also appears to contribute to downregulation of nephrin synthesis, an effect that would promote diabetic proteinuria.

Other recent work suggests that inhibition of the formation of glycated albumin, akin to pharmacologic AGE inhibition, may beneficially affect the development of diabetic nephropathy. In the db/db mouse model of diabetic nephropathy, treatment with an inhibitor of the formation of glycated albumin normalized glycated albumin concentrations, in association with decreased urinary albumin excretion and decreased urinary collagen IV [47]. The test compound also decreased renal expression of mRNAs encoding fibronectin and collagen IV and reduced mesangial matrix expansion.

Conclusions

Advanced glycation end product pathobiochemistry has become increasingly complex, and presents several opportunities for AGE inhibition. More is being discovered about the field of AGE inhibitors as new drugs proceed through regulatory trials. The few drugs in clinical development vary in their inhibitory actions, safety and efficiency profiles, and developmental strategies. Although progress has been made, the approval of an AGE inhibitor for clinical use for diabetic complications remains a challenge for the near future.

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