Therapeutic Interruption of Advanced Glycation in Diabetic Nephropathy

Do All Roads Lead to Rome?

KARLY C. SOURRIS, JOSEPHINE M. FORBES, AND MARK E. COOPER

JDRF Albert Einstein Centre for Diabetes Complications, Diabetes and Metabolism Division, Baker Heart Research Institute, Melbourne, Victoria, Australia

A major common feature of the chemically disparate compounds that inhibit advanced glycation end product (AGE) accumulation or signaling is their ability to show end-organ protection in experimental models of diabetes complications. The mechanisms by which these AGE- lowering therapies confer their benefits remain unsolved. Is it the reduction in tissue AGE levels *per se* or the inhibition of downstream signal transduction (as has been described with the soluble receptor for AGE)? Possible modes of action that need to be investigated include the ability of some of these agents to stimulate antioxidant defenses, to lower cholesterol and other lipid levels, and to inhibit low-grade inflammation. To understand these novel mechanisms, further examination of the advanced glycation pathway and, in particular, the diverse action of these agents in ameliorating the development of diabetic complications is needed.

Key words: **advanced glycation end products; diabetic nephropathy; reactive oxygen species; receptor for advanced glycation end products**

Introduction

In recent times there has been ongoing identification of novel inhibitors of nonenzymatic glycation as well as increased recognition that existing therapies generally considered to act on other pathways can also interrupt this biochemical process. Currently available therapies that have been noted to influence the advanced glycation pathway include thiazolidinediones,[1] blockers of the renin–angiotensin system,[2] and high-dose aspirin.[3]

Early inhibitors of advanced glycation, such as aminoguanidine (pimagedine)[4] and OPB-9195,[5] relied on direct scavenging of advanced glycation end product (AGE) precursors, such as pyridoxal acting to trap reactive carbonyl groups. The antidiabetic agent metformin can also trap reactive carbonyls in addition to lowering glucose (because of its guanidine moiety) and lowering circulating levels of reducing sugars.[6] Although carnosine[7] and aspirin[3] trap reactive carbonyls to reduce AGE formation, they also chelate copper and other transition metals, as has been reported for angiotensin-converting enzyme (ACE) inhibitors (ACEi) and angiotensin type 1 receptor (AT1R) antagonists,[8] and this chelation activity could possibly influence their ability to act as AGE inhibitors. Subsequently, putative AGE cross-link breakers, such as the prototype phenacylthiazolium bromide (PTB)[9] and a more stable derivative, alagebrium chloride,[10] were reported to cleave preformed AGEs. Furthermore, therapeutic benefits of lowering dietary intake of AGEs are also seen in diabetes complications.[11] Finally, B-group vitamins and derivatives (such as thiamine, benfotiamine,[12] and pyridoxamine[13]), which are potent inhibitors of advanced glycation, show many of the inhibitory mechanisms of action listed above.

These compounds appear to have diverse mechanisms of action and yet have many similarities, particularly with respect to their effects on downstream pathways. This review provides an overview of some of the pathways implicated in diabetic nephropathy, focusing on those agents that clearly lower AGE levels, particularly in tissues susceptible to diabetes-related injury (TABLE 1).

Pathways to End-organ Damage in Diabetic Nephropathy

It is likely that the damage seen in the diabetic kidney is the result of an interaction between hemodynamic and metabolic abnormalities,[14] as evidenced by the major clinical determinants of diabetic nephropathy,

Address for correspondence: Associate Professor Josephine Forbes, JDRF Albert Einstein Centre for Diabetes Complications, Baker Heart Research Institute, PO Box 6492, St Kilda Road Central, Melbourne, 8008, Australia. Voice: +61 3 8532 1456; fax: +61 3 8532 1288.
Josephine.forbes@baker.edu.au

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TABLE 1. A summary of advanced glycation end product (AGE)-lowering therapies with diverse mechanisms of action

Therapy	Tissue AGEs	Circulating AGEs
Carnosine	√	√
Benfotiamine	√	√
Thiamine	√	ND
Pyridoxamine	√	ND
Aminoguanidine	√	√
OPB-9195	√	√
ACE inhibitors	√	√
AT1 antagonists	√	X
Aspirin	√	ND
Metformin	√	√
Thiazolidinediones	ND	√
sRAGE	ND	ND
ALT-711 (alagebrium)	√	√

ND, not determined ACE, angiotensin-converting enzyme; AT1, angiotensin type 1 receptor; SRAGE, soluble RAGE.

hyperglycemia,[15] and hypertension.[16] Furthermore, these hemodynamic and metabolic pathways have been shown to interact in diabetic nephropathy.[17,18] FIGURE 1 represents a theoretical cascade of events which would likely result in the end-organ damage seen in the diabetic kidney.

Metabolic Links to AGE Inhibitors

A range of metabolic abnormalities, in addition to hyperglycemia, are seen in the diabetic kidney. However, it is obvious from studies in diabetic patients that glucose is the predominant metabolic abnormality in type 1 diabetes and strict glycemic control remains the critical, but often unattainable, strategy to retard the progression of nephropathy.[15,19] Both metformin and thiazolidinediones, which are agents that are widely used to improve glycemic control in type 2 diabetes, also influence tissue and circulating levels of AGEs.[20] Interestingly, a number of agents that may influence AGEs, such as ACEi and thiamine derivatives, have been reported to directly influence intracellular glucose uptake. Whether this ultimately leads to reduced intracellular AGE accumulation has not been extensively examined but should be considered. There is increasing evidence that intracellular AGEs and potentially AGE-binding proteins that are predominantly intracellular in location, such as the ezrin–radixin–moesin (ERM) proteins, may play a pivotal role in diabetic complications, such as nephropathy.[21,22] Indeed, the influence of other AGE inhibitors on glycemic control and cellular uptake of glucose has not been previously defined. For example, low AGE-containing diets have been reported to improve insulin sensitivity in models of type 2 diabetes[23] and in insulin-resistance states as occur with high fat feeding.[24]

Another metabolic abnormality characteristic of patients with type 1 and type 2 diabetes is hyperlipidemia, including hypertriglyceridemia and increased oxidized low-density lipoproteins.[25] The dyslipidemia in type 1 diabetes is not as overt as that seen in type 2 diabetes and most likely involves abnormal chemical modifications of lipoproteins with subsequent changes in biological function, rather than significant changes in lipoprotein levels. Interestingly, a number of the AGE inhibitors assessed in this review (TABLE 1) have been reported as improving lipid profiles in diabetic patients[26] and in experimental models of diabetic complications.[12,13,27] The relevance of this effect of end-organ proteins conferred by these agents remains to be elucidated, but reducing atherogenic lipids is likely to be clinically desirable in individuals with or at risk of diabetic complications.

The renin–angiotensin system (RAS) and in particular its hormonal vasoactivator peptide angiotensin II play a critical role not only in the regulation of systemic and glomerular hemodynamics but also in glomerular hypertrophy and ultimately glomerulosclerosis. Indeed, the therapeutic blockade of the renin–angiotensin system with either ACEi and AT1R antagonists remains a major component of therapies in both type 1 and type 2 diabetic patients with complications.[28,29] It should be appreciated that in addition to the agents that interrupt the RAS, other AGE-reducing agents, including pyridoxamine,[13] OPB-9195,[30] thiazolidinediones (mild), and carnosine,[7] have been shown to have direct hemodynamic effects, including reductions in systemic blood pressure.[16] This may not be a surprising finding since we have previously reported direct interactions between the advanced glycation pathway and the RAS. Specifically, the administration of exogenous AGE-BSA modulates the expression of various intrarenal components of the RAS with a pattern similar to that seen in the diabetic kidney.[18] Furthermore, both ACEi[17] and AT1R[31] decrease tissue accumulation of AGEs.

Downstream Effectors of Metabolic and Hemodynamic Pathways

There are four main downstream pathways that have been hypothesized to explain how glucose, through excess generation of reactive oxygen species (ROS), leads to the development of diabetic complications, including nephropathy. These also include increased flux via the polyol and hexosamine pathways, accumulation of AGEs, activation of protein kinase

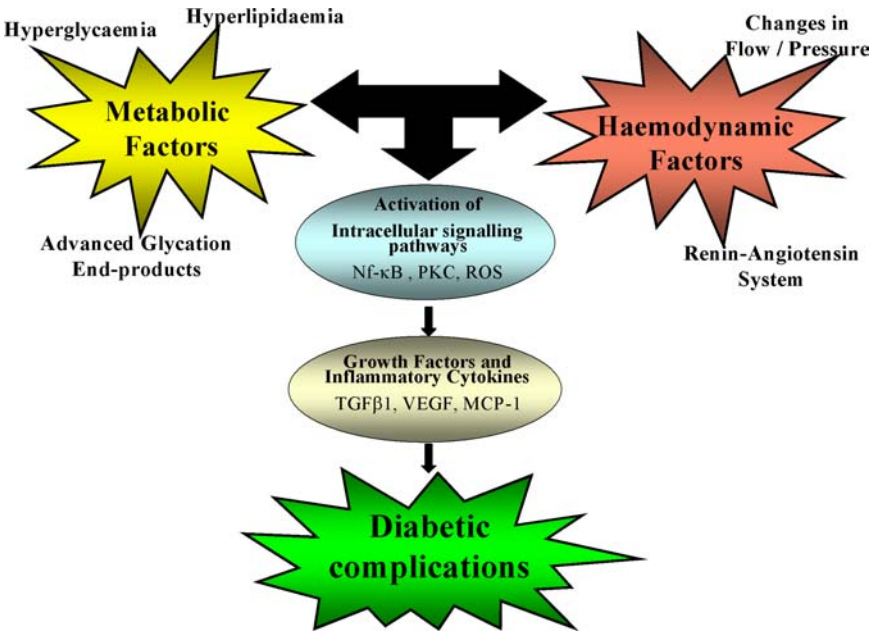

FIGURE 1. A theoretical cascade for the pathogenesis of diabetic complications: the downstream consequences of interactions between hemodynamic and metabolic pathways. (Adapted from Diabetologia Cooper 2001.[14])

C (PKC), or translocation of nuclear factor kappa B (NF-κB).[21] Indeed, therapeutic manipulation of each of these individual pathways has been shown to confer benefits in experimental models of diabetic complications. However, their individual contributions of modulating each of these pathways for the clinical management of diabetic nephropathy remains to be determined.

The excess generation of ROS as a result of hyperglycemia appears to enhance the progression of diabetic complications, with both cytosolic and mitochondrial sources of ROS being implicated. Indeed, the overexpression of cellular antioxidants, such as copper- or zinc-containing superoxide disumtase, protect against end-organ damage in models of type 2 diabetic nephropathy.[32] In addition, there have been many studies suggesting direct modulatory effects of AGEs on oxidative stress. The therapeutic approaches for reducing AGE accumulation and/or signaling discussed within this review have, in general, been reported to decrease ROS generation within complication-prone organs or in relevant cell culture experiments. It is likely, however, that the exact mechanism whereby each AGE inhibitor results in decreasing ROS generation may differ.[33] Furthermore, the involvement of specific cellular compartments, in particular the relative contribution of the mitochondrial versus cytosolic sources of ROS, remains to be determined.

Activation of PKC is considered to specifically activate a number of downstream signaling pathways in the pathogenesis of diabetic complications. Of particular relevance is the renoprotection afforded by blockade of the PKC-β1 isoform with LY333531[34] in experimental diabetic nephropathy. Furthermore, another PCK isoform, PCK-α, may also be involved since the genetic deletion of the PKC-α isoform in diabetic mice completely abrogates renal functional abnormalities.[35] Indeed, the majority of therapeutic agents described in this review (TABLE 1) prevent diabetes-induced activation and often membrane translocation of PKC-β1[12,36] or -α.[27] It should be noted that effects on PKC have not been determined in all the anti-AGE agents described in this review, and, therefore, it is possible that PKC inhibition is a common feature of agents which retard renal AGE accumulation.

The nuclear translocation of NF-κB by hyperglycemia has been demonstrated on many occasions in acute tissue culture experiments[37] and has also been confirmed in more experimental models of chronic diabetic complications.[38,39] However, it should be noted that when examined, AGE inhibitors do not appear to significantly influence diabetes-induced NF-κB translocation within renal tissues.[2,12,40]

The specific contribution of low-grade inflammation to a chronic disease, such as diabetic nephropathy, is increasingly being delineated. What is clear is that the blockade of specific cytokines and chemokines

involved in processes such as the recruitment of inflammatory cells, including monocyte chemoattractant molecule (MCP-1), appears to be a valid therapeutic strategy in models of diabetic nephropathy.[41] For example, blockade of the chemokine MCP-1 has been shown to attenuate not only macrophage infiltration but also progressive renal injury in *db/db* mice, a model of type 2 diabetic nephropathy. The link between AGEs and these chemokines has been evaluated by examining the reduction tissue expression of MCP-1 with a range of AGE-modifying drugs including, AT1 antagonists, aminoguanidine, aspirin,[42] soluble RAGE (sRAGE),[43] and thiazolidinediones.[1] Interestingly, each of the approaches described above that inhibit AGE accumulation or signaling of AGE-dependant pathways appears to be, in general, anti-inflammatory, although the specific cytokines that they modulate appear to vary among the different drugs.

Several *in vitro* and *in vivo* studies have implicated transforming growth factor-β (TGF-β), a fibrogenic cytokine, as a key effector molecule in promoting diabetic renal disease. To date, several anti-AGE therapies, including alagebrium,[10] ACEi, AT1 antagonists,[44] sRAGE,[45] aminoguanidine,[10] OPB-9195,[46] and aspirin,[42] have been shown to ameliorate diabetes-induced increases in either TGF-β1 or another profibrotic cytokine, connective tissue growth factor. The utility of TGF-β1 as a target for therapeutic intervention in diabetic nephropathy is hampered by its vital role in inflammatory and immune processes, and it may be preferable to suppress renal TGF-β1 levels by an alternative approach, such as therapies that focus on the advanced glycation pathway.

Other growth factors have been implicated in the progression of diabetic nephropathy, including angiogenic cytokines, such as vascular endothelial growth factor (VEGF).[47] One must, however, be cautious in the interpretation of all data since the suppression of VEGF or its receptors, particularly in the kidney, remains controversial with some studies suggesting that VEGF blockade will result in less albuminuria[48] and recent studies, albeit in a nondiabetic context, suggesting that VEGF is a critical renal survival factor and that blockade may promote renal damage.[49] This is best demonstrated by the diverse effects seen with anti-VEGF antibodies.[48] To date, within renal tissues, a specific decrease in VEGF expression in conjunction with improved renal functional and structural parameters has been seen with a range of AGE inhibitors, including alagebrium,[27] ACEi,[47] sRAGE,[45] and OPB-9195.[46]

Conclusion

Despite diverse chemical structures and a variety of different mechanisms of action, each of the strategies presented in this review that reduce accumulation of AGEs in tissues and/or relevant downstream signaling pathways, appear to confer their benefits via a number of common pathways. These anti-AGE therapies reduce cellular oxidative stress, decrease inflammation and macrophage infiltration, lower renal cytokine expression, and often alter serum lipid levels. These diverse biological actions were observed in the context of providing end-organ protection in a variety of models of diabetic complications. In addition, many of these agents reduce blood pressure and exhibit PKC activation (some 60%). Interestingly, only a few of these agents appear to have direct glucose-lowering effects, and effects on NF-κB activation are not generally observed. Importantly, however, current treatment strategies in clinical practice have little effect on lipid profiles, full-length RAGE expression, and cellular glucose uptake and compartmentalized mitochondrial production of superoxide. In conclusion, this review summarizes our understanding of the relative importance of multiple pathways of tissue damage implicated in the pathogenesis of diabetic complications and, in particular, the relationship of these pathways to AGE-lowering therapies that are currently being used, albeit for other reasons, or are in ongoing preclinical or clinical development.

Conflict of Interest

The authors declare no conflicts of interest.

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