

A New Perspective on Therapeutic Inhibition of Advanced Glycation in Diabetic Microvascular Complications: Common Downstream Endpoints Achieved Through Disparate Therapeutic Approaches?

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Key Words

Advanced glycation end products · Diabetic nephropathy · Reactive oxygen species · Receptor for advanced glycation end products

Abstract

A commonality among the chemically disparate compounds that inhibit the formation and accumulation of advanced glycation end products (AGEs) or their signalling pathways is their end organ protection in experimental models of diabetes complications. Although this group of therapeutics are structurally and functionally distinct with numerous mechanisms of action, the most important factor governing their therapeutic capability is clearly their ability to alleviate the tissue burden of advanced glycation, rather than the biochemical mechanism by which this is achieved. However, it remains to be determined if it is the reduction in tissue AGE levels per se or inhibition of downstream signal pathways which is ultimately required for end organ protection. For example, a number of these agents stimulate antioxidant defences, modify lipid profiles and inhibit low-grade inflammation. These novel actions emphasise the importance of further examination of the advanced glycation pathway and in particular the diverse action of these agents in ameliorating the development of diabetic complications such as nephropathy.

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Introduction

Hemodynamic and metabolic pathways induced by hyperglycaemia, are thought to mediate end organ damage in diabetic renal disease [1–4]. Indeed strict glycaemic control [5, 6] and blood pressure lowering therapies including those agents which provide blockade of the renin-angiotensin system [5, 7] remain the most successful clinically applicable therapeutic interventions for diabetic micro- and macrovascular complications.

One such metabolic pathway facilitated by the hyperglycaemic environment which is characteristic of diabetes is advanced glycation, where the free amino groups of proteins and amino acids are irreversibly modified as the result of complex non-enzymatic biochemistry [8]. Although physiologically, the accumulation of advanced glycation end products (AGEs) is mostly the result of aging and metabolism including senescent labelling of proteins, it is also common in modern food preparation and processing [9]. Therefore, it is postulated that both endogenously [10] and exogenously [10] derived AGEs directly contribute to the body's AGE pool.

Of particular interest is that both glycaemic control [11] and blockade of the renin-angiotensin system [12, 13] attenuate the accumulation of tissue AGEs, as do a number of agents which are often administered to diabetic patients including thiazolidinediones [14] and high-dose

aspirin [15, 16]. The anti-diabetic agent metformin can also lower circulating levels of reducing sugars [17, 18] and consequently AGEs.

Many of the pathogenic pathways known to mediate diabetic complications are associated with the accumulation of AGEs and/or activation of their downstream signalling pathways. Among these, hyperlipidaemia is often another metabolic characteristic of patients with diabetes [19, 20]. Furthermore, persistent hyperglycaemia also promotes a pro-oxidant environment, with a number of studies suggesting direct modulation and exacerbation of oxidative stress by AGEs [21]. In addition to oxidative stress, tissue-specific inflammation also occurs, most likely resultant from signalling molecules including those activated by specific AGE receptors. These include amongst others, nuclear factor- κ B [22], mitogen-activated protein kinases (MAPK) [23] and protein kinase C [24] which facilitate chemotaxis of leukocytes and the production of proinflammatory and profibrotic cytokines, all known to contribute to progressive diabetic microvascular complications.

Within the context of this review, we discuss the current therapies available for lowering or preventing the accumulation of AGEs and those which prevent downstream signalling from receptor ligation. We investigate why such disparate agents have many common downstream outcomes, which may contribute to their end organ protective properties in diabetes. It is prudent to suggest that future therapies for microvascular complications need to address these common points via specific targeting of sites downstream of advanced glycation, in particular those not affected by current clinical regimens such as RAS blockade.

Therapeutic Modulation of Advanced Glycation End Products

Advanced Glycation as a Specific Target for Diabetic Nephropathy

AGEs are a heterogeneous and complex group of modifications, which play an important role in the pathogenesis of diabetic nephropathy. AGEs are formed as a result of non-enzymatic biochemical reactions initiated as the Maillard reaction [25]. Studies in type 1 diabetic patients show that AGE accumulation predicts the severity of micro- and macrovascular complications. Specifically, serum AGE levels are significantly elevated with the progression to microalbuminuria and subsequently to overt nephropathy [26]. In addition, skin collagen-associated

AGE concentrations correlate with the severity of microvascular complications in patients with long-standing type 1 diabetes [11] and with carotid intimal thickening [27]. In type 2 diabetic patients, hypertension and ischemic heart disease are known to correlate with circulating AGE levels, suggesting that they may be potential biomarkers of diabetic cardiovascular risk [28]. Although AGE modifications are facilitated by the hyperglycaemic environment, they can be produced in other contexts. Indeed, some studies demonstrate that AGE levels only loosely correlate with glycaemic control in the clinical setting [11, 29]. This finding may explain the paradoxical progression of diabetic complications in some patients with comparatively good glycaemic control. Further to this, within the DCCT study, AGE levels were a better predictor of progression to complications than HbA_{1c}, with over a third of the variance in complications attributed to differences in AGE indices [11]. This is consistent with the hypothesis that other factors such as oxidative stress may contribute to the production and accumulation of AGEs in patients with good glycaemic control [30, 31].

There is no consensus on which AGE modifications are the most pathogenic in diabetes. Many of the AGE cross-linked moieties, such as pentosidine, have intrinsic fluorescence, and therefore tissue and plasma fluorescence may be used as surrogate markers of AGE modifications. A marked increase in fluorescence within the kidney [32], the retina [33], skin [34, 35] and other sites of diabetic microvascular disease [36] have been found with the progression of diabetes. Alterations in renal and hepatic function are also associated with increased tissue fluorescence, reflecting the role of these organs in the clearance of AGEs [37]. In addition, circulating levels of fluorescent AGEs correlate with complications in patients with type 1 and type 2 diabetes [38, 39].

Other AGEs, such as N-carboxymethyllysine (CML), which are neither cross-links nor fluorescent, have been found to be elevated within serum of type 1 diabetic patients [40]. Type 2 diabetic patients follow a similar pattern with increases in circulating CML [41] and the precursor dicarbonyl methylglyoxal [42]. Elevations in CML levels have been associated with the presence of microvascular complications, including retinopathy and nephropathy [43].

AGEs can elicit their effects via ligation to receptors. Receptors for AGEs may be loosely grouped as either inflammatory, such as the receptor for advanced glycation end products (RAGE) [44], or those involved in AGE clearance (e.g. AGE-R1 [45], AGE-R3 [46], CD36 [47] and

Table 1. Summary of AGE-lowering therapies with diverse mechanisms of action in diabetic complications

Therapy	Tissue AGEs	Circulating AGEs	Glycemic control	NF-κB activity	ROS	PKC/MAPK activity
Benfotiamine	√ ⁶⁹	√ ⁶⁹	√ ¹⁹	√ ¹¹⁶	√ ¹¹⁶	√ ¹¹⁶
Thiamine	ND	ND	√ ⁶⁹	√ ³¹	√ ³¹	√ ³¹
Pyridoxamine	√ ^{33, 67}	ND	ND	ND	√ ¹¹⁵	ND
Aminoguanidine	√ ⁵⁵	√	√	ND	√ ¹²¹	√ ¹²⁷
OPB-9195	√ ^{118, 180}	√ ¹⁸⁰	ND	ND	√ ^{83, 118}	√ ⁸³
ACE inhibitors	√ ⁶²	√ ^{13, 62, 111}	√ ¹⁰¹	√ ¹³	√ ¹³	√ ¹²⁷
AT1 antagonists	√ ⁶³	ND	ND	√ ¹³⁷	√ ¹³	√ ¹²⁹
Aspirin	√ ¹⁵	ND	ND	√ ^{96, 140}	√ ^{96, 122}	√ ¹²²
Metformin	√ ¹⁷	√ ⁶⁰	ND	√ ¹³⁰	√ ¹¹⁷	√ ¹³⁰
Thiazolidinediones	ND	√ ¹¹⁷	√	√ ¹⁴	√	ND
sRAGE	ND	ND	ND	√ ⁹²	√ ^{119, 159}	ND
Alagebrium chloride	√ ⁷⁹	√ ⁷⁹	ND	√ ¹¹¹	√ ¹¹¹	√ ¹⁰⁶
Carnosine	√ ^{73, 74}	√ ⁷⁴	ND	√ ¹⁴¹	√ ⁷⁴	ND

√ = Reported benefits with reference numbers; ND = not determined to date.

Scr-II [48, 49]). Vascular, renal, neuronal and haematopoietic cells are all known to express receptors for AGEs [45, 46, 50–54].

Therapeutics which Inhibit the Accumulation of AGEs

Numerous AGE inhibitors have been identified to date, which vary in their mechanisms of action and yet all have the same outcome, a reduction in tissue AGE burden. Some target the precursors of AGEs, thus preventing their formation and accumulation, whilst others target receptor signalling. Indeed, a number of inhibitors exhibit other benefits such as control of glycaemic indices and hyperlipidaemia in addition to reduction in AGE accumulation (table 1).

AGE Formation Inhibitors

Some of the earliest identified inhibitors of AGE-formation, such as aminoguanidine [55] and OPB-9195 [56] are known to reduce AGE accumulation by scavenging free reactive carbonyl groups [56–59]. More recently, novel therapeutics, such as the anti-hyperglycaemic agent metformin, trap reactive carbonyl molecules in addition to lowering blood glucose, properties most likely attributable to its guanidine moiety [60]. Aspirin has also demonstrated the capacity to decrease AGE accumulation by targeting preformed intermediates [15], by chelation of copper and other transition metals which contribute to ROS production, as well as scavenging free carbonyls.

Compounds which target the renin-angiotensin system, including angiotensin-converting enzyme (ACE) inhibitors and angiotensin type 1 (AT1) receptor antagonists, are the most effective treatments for diabetic nephropathy [7, 61]. Not surprisingly, these agents have shown that in addition to their hemodynamic actions, they have the added benefit of reducing AGE accumulation [12, 13, 62, 63]. ACEi and AT-1 antagonists decrease AGE accumulation by trapping reactive carbonyls, decreasing the formation of hydroxyl and carbon-centred radicals and also via chelation of metal ions [13].

LR-90 (methylene bis-[4,4'-(2-chlorophenylureido)phenoxyisobutyric acid]) has been identified as a potent inhibitor of renal and circulating AGE accumulation [64, 65]. It is thought to reduce AGE accumulation via its potent metal chelating abilities and its interaction with reactive carbonyl species [65]. Moreover, its renoprotective benefits, such as improvements in albuminuria, creatinine clearance and glomerular sclerotic index, have been demonstrated in experimental models of both type 1 and type 2 diabetic nephropathy [64, 65].

The benefits of vitamin B compounds in lowering AGE accumulation have been extensively investigated. Pyridoxamine, a vitamin B₆ derivative, prevents the formation of AGEs from Amadori intermediates [58, 59] in addition to cleavage of 3-deoxyglucosone-reactive carbonyl intermediates [66]. Not surprisingly, the inhibitory actions of pyridoxamine on AGE accumulation are associated with concurrent improvements in renal function

in experimental models [67]. More importantly, in phase II studies in patients with diabetic renal disease, a treatment effect was observed on the rise in serum creatinine with pyridoxamine [68].

Thiamine and benfotiamine are liposoluble derivatives of vitamin B₁ which also exhibit AGE-lowering properties. In contrast to pyridoxamine, benfotiamine and thiamine are known to decrease the formation of reducing sugars and intermediates from the polyol pathway [69]. Both benfotiamine and thiamine have proven to be beneficial in experimental models of diabetic nephropathy [31, 70]. Furthermore, administration of benfotiamine to type 2 diabetic patients, who consumed a diet high in AGE content, reduced circulating AGE levels and markers of oxidative stress [71]. However, another study has demonstrated a lack of effect of benfotiamine on complication-causing pathways in type 1 diabetic patients [72].

Carnosine is a naturally occurring dipeptide which is known to exist in the brain as well as many other tissues. Importantly, it has a number of activities which aid in the reduction of AGE accumulation. Specifically, in addition to its anti-oxidant properties, carnosine also reacts with aldehydes such as aldose and ketose sugars preventing AGE formation [73–76]. Its protective benefits have been demonstrated in models of experimental diabetic nephropathy [77].

Cleavage of Pre-Formed AGEs

Advanced glycation products form non-reversible covalent cross-links within and between tissue proteins and other organic compounds. Novel therapeutics such as N-phenacylthiazolium bromide (N-PTB) [78] and alagebrium chloride (ALA) [79] cleave cross-links, allowing for the clearance of glycated proteins via scavenger receptors and renal excretion. The prototype N-PTB [78] had the ability to cleave α -dicarbonyl intermediates in the AGE formation pathway, as was shown in tail collagen experiments [78]. Apparent toxicity and unexplainable increases in blood pressure seen with PTB meant that it was not a realistic therapeutic for development in humans [80]. A more stable thiazolium derivative, ALA [79], was reported to catalytically cleave pre-established AGE cross-links between proteins, although the exact mechanisms of action of ALA remain to be fully determined. Alagebrium has proven to have utility in human isolated systolic hypertension and diastolic heart failure [81].

Blockade of Cellular Receptors of AGEs

Binding of AGEs to proteins such as the receptor for advanced glycation end products (RAGE) has been shown

to mediate intracellular signalling pathways, modulate gene expression and accelerate inflammation [82]. Double transgenic mice which over-express RAGE in addition to being deficient in iNOS (inducible nitric oxide synthase) exhibit renal hypertrophy, albuminuria, and elevated serum creatinine relative to their wild type mice [83].

Soluble forms of RAGE produced either from alternative gene splicing (esRAGE) [84] or by proteolysis (sRAGE) [85, 86] show promise as a possible future therapeutic. Plasma levels of sRAGE have been shown to be lower in type 1 diabetic patients [1, 87] and elevated in type 2 diabetics. Indeed, with the development and progression of diabetic nephropathy, changes in circulating sRAGE concentrations are thought to be independently associated with renal function [88–91]. In an experimental model representative of type 2 diabetic nephropathy, administration of sRAGE was shown to diminish albuminuria and improve glomerulosclerosis [92].

Thiazolidinediones (TZDs) are known ligands for peroxisome proliferator-activated receptor-gamma (PPAR- γ), which exhibit a number of beneficial actions in diabetic nephropathy independent of insulin sensitization. TZDs, specifically rosiglitazone, have recently been identified as RAGE antagonists [14, 93, 94]. Interestingly, rosiglitazone administered to type 2 diabetic subjects increases soluble RAGE concentrations in addition to decreasing circulating AGE levels [95].

Metabolic Disturbances

Abnormalities in Glycaemic Control

A range of metabolic abnormalities in addition to hyperglycaemia are seen in the diabetic milieu. However, it is obvious from studies in diabetic patients that elevations in circulating glucose are the predominant metabolic abnormality and strict glycaemic control remains the critical but often unattainable strategy to halt the progression of complications [5, 6]. As well as promoting the formation of AGEs, chronic hyperglycaemia is also associated with increased inflammation and expression of associated inflammatory cytokines, such as MCP-1 [96], and connective tissue growth factor (CTGF) [97]. In spontaneously diabetic rats strict glycaemic control inhibited the formation of pentosidine in renal tissue, compared with hyperglycaemia which promoted AGE accumulation and associated complications [98]. Metformin, an insulin sensitiser, both lowers serum glucose levels and inhibits the formation of AGEs [60, 99]. Combination

therapy of metformin with either pioglitazone or rosiglitazone resulted in improved long-term glucose handling [100]. However, the influence of other AGE inhibitors on glycaemic control as well as cellular uptake of glucose has not yet been defined. Similar results in retinal cells using captopril [101] also showed a reduction in glucose-mediated damage via GLUT-1 (membrane glucose transport protein) and the results of both these studies appear to be insulin-mediated occurrences.

Hyperlipidaemia

Hyperlipidaemia is a comorbidity often seen in diabetic patients and is thought to be an important contributor to progressive micro- and macrovascular complications. This is most clearly demonstrated by the renoprotection which is seemingly afforded with HMG CoA reductase inhibitors [102–104]. Interestingly, the majority of the AGE inhibitors assessed in this review (table 1) have been reported to improve lipid profiles in diabetic patients [99], as well as in experimental models of diabetic complications [31, 33, 64, 67, 69, 79, 105, 106]. Effects of RAS blockers, sRAGE and TZDs in plasma lipids have not been previously reported. It remains to be determined as to the specific mechanism by which these benefits on hyperlipidaemia are conferred. To date, none of these therapies, however, has been shown to increase plasma concentrations of HDL, although effects on reverse cholesterol transport have been recently highlighted with alagebrium, aminoguanidine [107], pyridoxamine and metformin [108].

Effects of AGE Inhibition on Hemodynamic Pathways

The UK prospective diabetes study in type 2 diabetic patients highlights the importance of hemodynamic influences in the development of DN. Diabetic subjects randomised to receive tighter blood pressure control exhibited a concomitant reduction in microalbuminuria and clinical proteinuria [5]. To date the most effective treatments for both type 1 and type 2 diabetic patients to retard the progression of diabetic complications are anti-hypertensives which target the renin-angiotensin system [7, 109]. Studies in the Ren-2 rat, a strain with genetic overactivity of the RAS, show amelioration of renal injury following treatment with a selective inhibitor of AGE formation, ALT-946, which is an aminoguanidine derivative [3]. In addition, infusion of AGEs into healthy rats induces diabetic-like changes in the renal RAS [4], suggesting that advanced glycation can modulate the RAS [110].

Benefits on the hemodynamic system, primarily via changes in blood pressure, have been exhibited by a num-

ber of other therapeutics which also reduce the accumulation of AGEs including alagebrium [79, 111], pyridoxamine [67], OPB-9195 [112], TZDs and carnosine [113].

Reactive Oxygen Species

Reactive oxygen species are important intermediates in the formation of AGEs and are often excessively generated in the kidney in diabetes [114]. In addition, concomitant dysregulation of anti-oxidant enzymes in diabetes leads to a state of oxidative stress [114]. To date, it is unclear as to why antioxidants per se have demonstrated such poor renoprotection in humans, despite exciting positive preclinical research findings; however, it seems evident that therapies such as vitamins may not be the ideal antioxidant strategy in human DN. Most of the inhibitors listed in table 1, including vitamin B₆ derivatives [115, 116], metformin [117], OPB-9195 [112, 118], ACEi [13, 111], AT1 antagonists [13], ALA [111] and sRAGE [119], have exhibited beneficial effects on excess superoxide generation within tissues, associated with improvements in the development and/or progression of diabetic complications.

Vitamin B-related therapeutics are effective scavengers of ROS intermediates. Pyridoxamine inhibits superoxide radical generation as well as preventing the progression of neuropathy and retinopathy [120]. In addition, benfotiamine and thiamine, vitamin B₁ derivatives, have shown beneficial effects in normalising ROS production and reducing the activity of aldose reductase [69].

Our group has shown that induction of diabetes results in a 50% increase in mitochondrial ROS production when kidneys were examined ex vivo. Treatment with alagebrium in this STZ rat model resulted in attenuation of both mitochondrial and cytosolic superoxide production [106]. Furthermore, glycation of mitochondrial proteins in diabetic rat kidneys has been associated with excess ROS production, inducing abnormalities in the mitochondrial respiratory chain complexes [121] which were prevented with administration of aminoguanidine [121]. Aspirin has also been shown to decrease reactive oxygen species production, in addition to increasing the production of NO in in vitro experiments [96, 122].

Inhibition of Protein Kinase C (PKC) Activity by AGE Inhibitors

There has been a growing body of evidence suggesting the central role of PKC, which is broadly involved in signal transduction from the plasma membrane to the nu-

cleus in diabetes-induced vascular dysfunction [123, 124]. PKC has 11 different isoforms many of which have been shown to be involved in diabetic complications in particular nephropathy [24, 125, 126].

From all of the therapeutics targeting advanced glycation presented in this review, some 60% have been identified as having direct effects on PKC. We have recently reported the attenuation of PKC- α phosphorylation and translocation with ALA in both in vivo models of DN and in vitro studies [106]. It remains to be determined if this action of alagebrium on PKC- α phosphorylation partly explains its renoprotective actions. Modulation of PKC activity within the diabetic kidney has also been exhibited by various vitamin B derivatives [31, 116]. Interestingly, both ACEi and aminoguanidine prevent diabetes associated increases in PKC- β activation in renal glomeruli [127].

The effects of aminoguanidine and ACEi on PKC- β activity were also observed at other sites of vascular injury including the retina and mesenteric vascular bed [13, 128]. In addition, AT-1 receptor antagonists also attenuate diabetes-induced increases in PKC-epsilon activity within the diabetic heart [129]. Furthermore, modulation of PKC has been demonstrated in vascular endothelial cells with the insulin-sensitizing agent metformin [130] and the anti-thrombotic therapeutic aspirin [122]. Hence, once again we encounter the multi-faceted pathways which are affected by blockade of advanced glycation or AGE-mediated signalling pathways.

Nuclear Transcription Factor Kappa-B (NF- κ B)

NF- κ B is a transcription factor composed of two subunits, the most common of which are the p50 and p65 subunits [131] which are thought to be important modulators of diabetic complications. The active p65 subunit, in particular, is thought to be central in the activation of numerous genes including cytokines, adhesion molecules, NO synthase, angiotensinogen and many other inflammatory and proliferative proteins implicated in the process of diabetic nephropathy [22, 131]. NF- κ B is activated by a range of stimuli including glucose [132] and ROS [133]. AGEs are also involved in activation of NF- κ B via a RAGE-dependent pathway leading to its translocation to the nucleus where it induces transcription of target genes such as IL-6 and TNF- α [134]. The diverse actions of NF- κ B and the capacity of various factors such as angiotensin II (AII) and AGEs to activate this transcription factor [135, 136] are consistent with NF- κ B playing a pivotal role in the pathogenesis of diabetic complications.

Pyrrolidine dithiocarbamate (PDTC) is a NF- κ B inhibitor which has been used in both diabetic [137] and non-diabetic animal models of renal disease where it is renoprotective [138], although its toxicity does not allow for direct translation to the clinical setting. As with ROS and PKC, a number of currently available AGE-modulating therapies, almost 40% have been shown to affect NF- κ B activation and translocation. Indeed, our group has demonstrated the importance of NF- κ B in the pathogenesis of early renal macrophage infiltration in experimental diabetes, which could be modulated by interruption of the RAS [137, 139]. Moreover, diabetes-induced increases in NF- κ B activation are prevented by numerous therapeutics including metformin [130], aspirin [140], vitamin B derivatives [116], carnosine [141], and thiazolidinediones [14]. It is possible that NF- κ B, like PKC, is a central mediator which drives the downstream pathogenic consequences of interactions between hemodynamic and glucose-dependent pathways in diabetic vascular complications. However, approaches to inhibit NF- κ B have not been explored fully in DN, most likely due to the intimate involvement of this transcription factor in a number of essential cellular processes including apoptosis.

Inflammatory Cytokines and Growth Factors

Diabetic nephropathy was not traditionally considered to be an inflammatory condition; however, there is a growing body of evidence in recent times highlighting the central role of inflammation in its development and progression [79, 142–150]. Indeed, both hemodynamic and metabolic factors involved in the development of diabetic complications such as nephropathy activate common downstream targets, including cytokines and growth factors [151]. In particular, monocyte chemoattractant protein (MCP-1), transforming growth factor- β 1 (TGF- β 1), CTGF and vascular endothelial growth factor (VEGF) have all been implicated in both experimental and human studies to be involved in the development and progression of diabetic nephropathy.

Monocyte Chemoattractant Protein-1 (MCP-1)

MCP-1 is a potent chemokine which encourages monocyte/macrophage infiltration into the kidney, which likely contributes to the progression of DN. MCP-1 production and secretion from damaged renal cells in diabetes are postulated to be responses to hyperglycaemia subsequently activating a number of signalling pathways including those mediated by PKC and NF- κ B [152]. Moreover, AGEs have also been identified as a specific stimulus for the production of MCP-1 [152, 153] and are

secreted by mesangial, epithelial and glomerular podocytes [146, 152]. In an experimental model of type 2 diabetic nephropathy, a deficiency in MCP-1 resulted in a significant reduction in renal inflammatory infiltration and renoprotection. Furthermore, administration of propagermanium, an antagonist of the MCP-1 receptor, in a model of diabetic nephropathy resulted in reduced renal hypertrophy and macrophage infiltration in renal glomeruli [154]. Indeed, it has been demonstrated that elevations in urinary excretion of MCP-1 may be a valid diagnostic marker of diabetic nephropathy in type 2 diabetic patients [155]. These studies suggest that MCP-1 is a central mediator of diabetic renal disease, although its utility as a therapeutic target remains to be determined [147].

Interestingly, many of the treatments which inhibit AGE accumulation or AGE-dependent signalling appear to be anti-inflammatory, although the specific cytokines which they affect appears to vary. To date, improvements in tissue MCP-1 expression are seen with a number of AGE inhibitors such as AT-1 antagonists [156], aminoguanidine [157], aspirin [97, 158], sRAGE [159] and thiazolidinediones [14], all of which are known to modulate other pathways.

Modulation of Growth Factors

Growth factors such as TGF- β , a fibrogenic cytokine, and CTGF, which is primarily induced by TGF- β 1, have been implicated as key effector molecules which promote diabetic renal disease. TGF- β is a superfamily with three mammalian isoforms. The major isoform, TGF- β 1, is synthesised as an inactive or latent form, which subsequently is subjected to proteolytic cleavage leading to the generation of the active form. TGF- β 1 binds to the type II receptor and subsequently binds to the type I receptor [160] inducing phosphorylation and intracellular signalling involving the SMAD proteins [161]. In vitro studies have shown that a range of stimuli increase TGF- β 1 expression including hyperglycaemia, AGEs, stretch, AII, endothelin, lipids and various products of oxidative stress such as F₂ isoprostanes, all factors relevant to DN [162–167]. Ziyadeh's group has previously examined the effects of long-term administration of a neutralizing TGF- β 1 antibody on renal function and structure in diabetic *db/db* mice [168] and STZ diabetic mice [169]. Although most of the benefits have been attributed to TGF- β 1, Hill et al. [170] suggested that another isoform, TGF- β 2, is closely linked to fibrogenesis in diabetic nephropathy. To date, several anti-AGE therapies including alagebrium [79], AT1 antagonists [171], sRAGE [92], aminoguanidine

[172], OPB-9195 [118] and aspirin [97] have been shown to ameliorate diabetes-induced increases in TGF- β 1. The utility of TGF- β 1 as a target for therapeutic intervention in DN, however, is impeded by its essential role in inflammatory and immune processes. Therefore, it may be preferable to modulate renal TGF- β 1 levels by an alternative approach such as therapies which focus on upstream advanced glycation pathways.

Connective Tissue Growth Factor

Another pro-sclerotic cytokine, CTGF, has increased renal [173, 174] and, in particular, glomerular expression in diabetes [173, 175] and elevated both in early and late diabetic nephropathy in humans [176]. Currently, a phase II study of FG-3019, a humanised anti-CTGF antibody, has been completed in patients with diabetic nephropathy (microalbuminuria) which was well tolerated and improved albuminuria. Subsequent studies are planned in diabetic patients with macroalbuminuria (<http://www.fibrogen.com/trials>).

CTGF expression is thought to be mediated by a number of factors common in diabetic nephropathy including TGF- β 1, hyperglycaemia and mechanical stretch [173]. Interestingly, AGEs have been reported to specifically increase CTGF expression, initially in fibroblasts [177] but subsequently in mesangial cells [177]. Indeed within our own study in STZ-induced DN, the AGE inhibitor aminoguanidine ameliorated renal increases in CTGF [174]. Moreover, aspirin has also been shown to prevent the diabetes-mediated increase in CTGF and mesangial expansion in experimental models of DN [97]. Currently, effects of other AGE-modifying regimens on CTGF have not been fully elucidated.

Vascular Endothelial Growth Factor

VEGF is a cytokine whose major role in diabetes was initially considered to be central for the pathogenesis of diabetic retinopathy and in particular retinal neovascularisation. Recent findings, however, have demonstrated the importance of VEGF within the diabetic kidney [92, 106, 118, 178, 179]. We and others have previously shown both in vivo and in vitro decreases in VEGF expression with a number of AGE inhibitors including alagebrium [106], ACE inhibitors [106], sRAGE [92] and OPB-9195 [118]. Despite this suppression of VEGF as a result of current therapeutics, the benefits of VEGF suppression remain controversial with some studies suggesting that VEGF blockade is renoprotective [179], whereas recent studies, albeit in a non-diabetic context, suggest that VEGF is a critical renal survival factor and that blockade

may in fact promote renal damage [180]. This is perhaps best demonstrated by the differential effects seen with anti-VEGF antibodies [192, 193]. Studies on the renal effects of blockade of VEGF receptor (VEGFR) signalling are currently being performed. Indeed, a recent preliminary report has shown that SU5416, a VEGFR tyrosine kinase inhibitor, reduces albuminuria in *db/db* mice [181]. In experimental models of DN, VEGF expression is also decreased by an inhibitor of AGE formation [182] and with the AGE cross-link breaker ALA [106] further confirming the link between AGEs and VEGF expression.

Conclusions

Despite diverse molecular structures and varied mechanisms of action, each of the strategies reviewed here with effects on the tissue accumulation of AGEs and/or relevant signalling pathways appear to confer their end organ protective benefits via a number of common downstream

pathways. Almost all of these anti-AGE therapies reduce cellular oxidative stress, decrease inflammation and improve circulating lipid profiles. These shared effects were observed in the context of providing end organ protection in a variety of models of diabetic complications. In addition, many (some 60%) of these agents reduce blood pressure and activate protein kinase C activation. Interestingly, only a few of these agents appear to have direct glucose-lowering effects, and effects on NF- κ B activation were not generally observed. Importantly, however, current treatment strategies which target the RAS in clinical practice have little effect on lipid profiles, full-length RAGE expression, cellular glucose uptake and compartmentalised mitochondrial production of superoxide. In conclusion, this review extends our understanding of the relative importance of AGE-mediated pathways in the pathogenesis of diabetic complications and, in particular, the common downstream events which warrant further investigation as therapeutic targets in ongoing preclinical or clinical development in addition to anti-AGE therapies per se.

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