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Review

RAGE: A Novel Target for Drug Intervention in Diabetic Vascular Disease

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At high levels as seen in diabetes, glucose reacts with and forms adducts (advanced glycation end products; AGEs) on macromolecules including proteins and DNA, eliciting cellular dysfunction and leading to vascular disease. The major means is through cellular receptors; the best characterized is the receptor for advanced glycation end products (RAGE). Accumulation of both AGE/RAGE in addition to other identified ligands of RAGE, including S100/calgranulins, is the hallmark of this receptor in disease pathogenesis. Blockade of ligand-receptor interaction directly at the protein level, or transgenetically, prevents development of micro vascular (nephropathy) and macro vascular (atherosclerosis/restenosis) disease in small animal models. Furthermore, allelic variants of RAGE exist that alter the protein function and gene expression, which may further affect disease outcome. In conclusion, RAGE is a target for drug development to prevent vascular disease in diabetic and nondiabetic subjects.

KEY WORDS: advanced glycation end products; diabetes; immunoglobulin receptor; nephropathy; polymorphism; restenosis; vascular disease.

GLYCATION OF MACROMOLECULES: A CYTOTOXIC PROCESS

The formation and subsequent impact of nonenzymatically glycated proteins has been shown to play a role in the development of a variety of pathogenic processes involving inflammation, renal failure, and, most importantly, diabetic complications secondary to hyperglycemia and oxidative stress. Although this is a relatively new accepted mechanism for eliciting disease, the underlying premise was first described by Louis Camille Maillard in 1912, who demonstrated that a nonenzymatic process was initiated when glucose was heat-incubated with various amino acids to yield a solution that was brown and pigmented (1). The *in vivo* occurrence of this process was demonstrated when chromotagenic analysis of hemoglobin subtypes isolated from diabetic patients identified a glycated product of hemoglobin A and glucose, termed HbA_{1C} (2). The clinical relevance of this process was shown, as these glycated hemoglobin isoforms were found to be elevated in diabetic subjects. Measurement of these species is now used as a retrospective measure to monitor long-term glucose control in patients (3).

Common to all types of diabetes are specific complications of the microvasculature (retinopathy, nephropathy, and neuropathy) and macrovascular disorders that are not dissimilar to those seen in the normal population. These occur with an increased prevalence and severity in diabetic subjects. The importance of glycation itself in the pathogenesis of these vascular diseases has been revealed by large prospective studies demonstrating that for every 1% increase in HbA_{1C} levels, a 37% increase in incidence of microvascular disease was seen (4).

At the molecular level, the nonenzymatic glycation process occurs due to the reactive nature of aldose sugars with amino groups of proteins to form a Schiff base (5). This undergoes rearrangements to form a complex structure known as an advanced glycation end product, or AGE (6). This differs from the classical enzymatic N- and O-linked glycosylation of proteins, as first, AGEs are irreversible modifications, and second, AGEs may alter the function of a protein, in contrast to N and O glycosylations, which dictate the protein's location in the cell and its function (7).

Initial studies on AGEs focused on their slow formation on long-lived proteins such as collagen (8), which occurs naturally with aging and at an accelerated level under conditions of hyperglycemia seen in diabetes and in inflammatory/prooxidative environments such as atherosclerosis (9). Recent studies indicate that AGEs not only form slowly on extracellular proteins, but also rapidly intracellularly from intermediates of glucose metabolism (10). The predominant AGE structure occurring *in vivo* is carboxy(methyl)lysine (CML) adduct, found to be elevated in diabetic subjects with renal failure and vascular disease (11). A variety of structures have been detected in tissues including pentosidine, pyrraline, and imidazolones (Fig. 1).

AGEs elicit their effect by a number of mechanisms. First, AGEs were implicated in the pathogenesis of vascular disease by their ability to cross-link proteins of the vascular vessel wall, altering their structure and function leading to vessel thickening and vascular leakage (12). The use of AGE inhibitory compounds in animal models blocked these effects,

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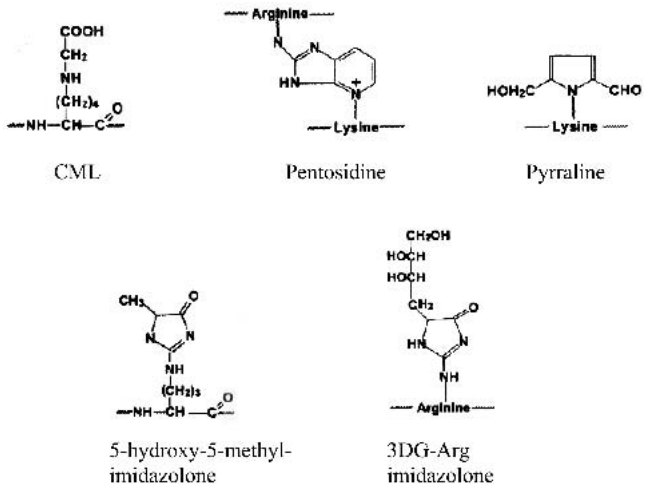

Fig. 1. Structures of common AGEs identified [adapted from Thorpe and Baynes (73)].

suggesting AGE inhibition as a mechanism of drug intervention in vascular disease (12). Second, rapid intracellular AGE formation can alter cellular function by changing protein structure and function. Third, AGEs have been shown to interact with specific cellular receptors that internalize and degrade AGEs or, importantly, trigger signal transduction pathways resulting in the activation of proinflammatory and procoagulant pathways. A number of receptors have been identified to date, which include the AGE-receptor complex (galectin-3, OST-48, and 80K-H) (13), macrophage scavenger receptors (type I and II) (14), LOX-1 (15), CD-36 (16), and the receptor for advanced glycation end products, or namely RAGE (17). Apart from their ability to bind highly glycated *in vitro*–produced albumin, the role for the majority of these receptors in the biology of AGEs and the related pathologies is unclear. However, numerous studies have clearly demonstrated the role of RAGE as a signal transduction receptor for AGEs prepared both *in vitro* and isolated from diabetic subjects, amplifying the effects of AGEs.

RAGE: A MULTILIGAND RECEPTOR

RAGE was first identified from bovine lung as a 35-kDa protein that bound AGEs in a dose-dependent manner (17). Cloning of RAGE and subsequent homology analysis revealed it to be a member of the immunoglobulin superfamily of receptors, comprising an extracellular domain consisting of a single V-type immunoglobulin domain and two C-type immunoglobulin domains (17). Molecular studies identified that the ligand binding site existed within the V-domain by performing binding assays with truncations of the extracellular domain (18). After the extracellular domain, there follows a single transmembrane domain and a short 43 amino acid cytosolic tail. The latter is highly charged and essential for RAGE function, as deletion of this domain imparts a dominant negative (DN) effect upon ligand engagement in both *in vitro* and *in vivo* studies (19). RAGE was first proposed to act as a scavenger receptor for AGEs on macrophages; however, further studies demonstrate RAGE to act predominantly as a signal transducer after binding AGEs to up-regulate gene expression (20). RAGE can induce a range of signal transduction pathways including Ras pathways (21) (stress response, apoptosis), Rac/Cdc42 (22) (cell growth and motility), and Jak/Stat pathways (23) (alteration of gene expression) (Fig. 2). However, the exact bridging molecule(s) that engage the RAGE tail are yet to be elucidated.

RAGE was found to be present in low levels in most tissues except lung, where strong message and antigen levels are detectable (24). RAGE was also found to be present at high levels in the developing embryonic rat brain; however, it is not thought to be a gene crucial for neuronal development, as RAGE knockout mice develop normally, live a natural life span, and are fertile (23). At the cellular level, RAGE is expressed by a variety of cell types, including endothelial, vascular smooth muscle, podocytes, and neuronal cells (24). Studies revealed a striking feature of RAGE expression; under proinflammatory conditions seen in a variety of diseases including diabetic vascular disease, RAGE expression is found to be highly up-regulated (24). Another remarkable characteristic of RAGE is that it's biological impact was

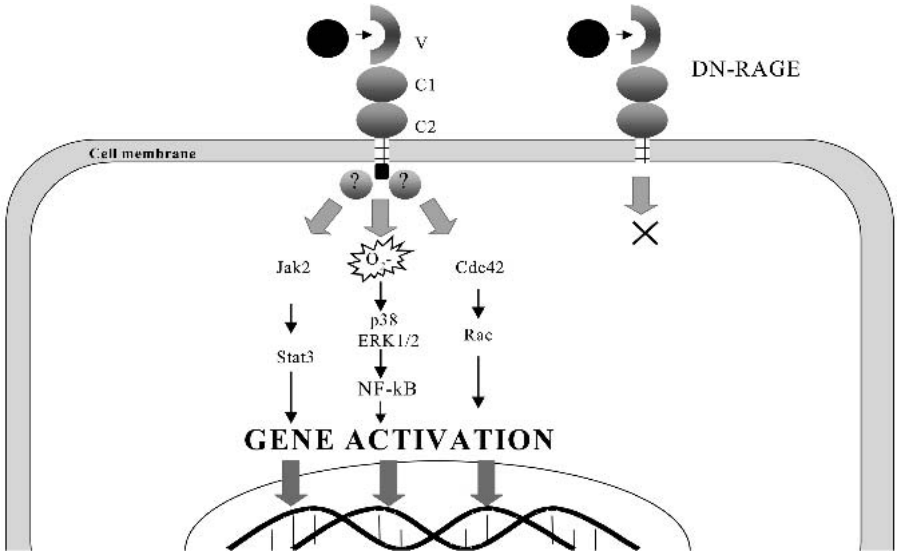

Fig. 2. Signaling pathways activated by RAGE/ligand interaction.

found to extend beyond the concept of a receptor for glycated proteins. Based on the observation of the high expression pattern of RAGE in lung, an environment in which low levels of AGEs are present, we investigated this tissue for the presence of endogenous physiological ligands. A number of interacting proteins were identified that included the amyloid beta peptide (Aβ) that forms fibrils central to the pathogenesis of Alzheimer's disease and a variety of amyloidoses (25). RAGE also binds amphoterin, also known as HMG-1 (high mobility group-1), a protein involved in neurite outgrowth and linked to tumor invasion (26). Most recently, RAGE was found to bind to a variety of the S100/calgranulin family of proteins, including S100A12 (extracellular newly identified RAGE-binding protein, or EN-RAGE) and S100B (19). This protein family consists of at least 15 proteins, their hallmark being the up-regulation in inflammatory disease settings (27). The fact that RAGE can bind multiple ligands is consistent with both the heterogeneous nature of AGE structures and the multiligand nature of other immunoglobulin receptors such as CD36 (28). The precise model of structural interaction between RAGE and its ligands have not been elucidated yet. However, insights from Igor Bronstein's group have demonstrated the successful crystalization of S100A12 and its functionality in these states (29). It was shown that S100A12 forms a hexameric assembly, and it was proposed this interacts with three RAGE extracellular domains in a trimeric assembly to activate signaling (29).

These observations led us and other groups to investigate the underlying biology and cell type expression of RAGE in normal homeostatic and pathogenic settings. Initial studies of samples from diabetic subjects with vascular disease and their equivalent rodent models demonstrated that not only was RAGE expression increased, but also the accumulation of at least two ligands—AGEs and S100/calgranulins—was enhanced (30,31). In a mouse model of diabetes-accelerated atherosclerosis, not only were AGEs up-regulated but also S100A12 in conjunction with increased RAGE expression (32). This led us to test the hypothesis that RAGE could be a factor in the development of diabetic vascular disease and that interrupting this receptor-ligand interaction may prove to be a valuable therapeutic tool.

RAGE AND VASCULAR DISEASE

The hallmark of vascular disease of diabetes and the first measurable progressive pathogenic step is the hyperpermeability of the vascular endothelium (32). To investigate this, our first *in vivo* studies of RAGE focused on a rodent model of this complication (33). Diabetes was induced in animals by streptozotocin, and, after 11 weeks of hyperglycemia, vascular leakage was demonstrated by measurement of tissue-blood isotope ratio (TBIR) (33). Both normal and diabetic rats were infused with *in vitro*–produced AGEs (AGE-BSA) or *in vivo*–produced AGEs isolated from human diabetic subjects. Injection of both forms of AGEs into rats resulted in decreased clearance and substantial vascular leakage of AGEs. To determine the role for RAGE in this process, RAGE-ligand interaction was blocked by two means. First, polyclonal anti-RAGE antibodies were injected, and second, the soluble extracellular domain minus the transmembrane domain and tail (sRAGE), previously demonstrated to bind ligands of RAGE, was injected (33). Administration of anti-RAGE IgG or sRAGE (2.25 or 5.15 mg/kg) strongly blocked the increase in vascular permeability in diabetic animals (33). In addition, blockade of RAGE could also reverse vascular hyperpermeability in diabetic rats treated 11 weeks after induction of diabetes. This therefore suggested that vascular hyperpermeability was not only reversible, but also indicated the role for RAGE and its blockade in established diabetic vascular disease. To test this hypothesis, we used a rodent model of accelerated diabetic atherosclerosis (34). Macrovascular disease is by far the most common complication to affect human diabetic subjects, accounting for around 70% of their mortality (35,36). Using apoE null mice made diabetic with streptozotocin, sRAGE treatment suppressed accelerated aortic lesion development in a glucose- and lipid-independent manner (34). Further assessment of ligand levels after treatment revealed that in the tissue and plasma, levels of AGE and S100 were decreased (34).

As atherosclerosis in human subjects is a progressive disease spanning many decades, the clinical relevance of these findings was extended to animals with established atherosclerosis (37). In order to verify these findings, apoE null mice were made diabetic at the age of 6 weeks and treated with sRAGE or placebo from the age of 14 to 20 weeks. With sRAGE treatment, mean lesion size and complexity was significantly reduced compared to placebo-treated animals (37). At the cellular level, sRAGE administration blocked the migration and proliferation of smooth muscle cells (SMC) and mononuclear phagocytes (37). Investigation of the underlying molecular mechanisms revealed sRAGE not only reduced RAGE expression but also CML-AGE accumulation and S100 expression. A further observation in these experiments was the effect of sRAGE on euglycemic nondiabetic atherosclerosis (37). It was found that sRAGE was able to reduce atherosclerotic lesion area and complexity in apoE null mice not rendered diabetic. This further supports the observation that RAGE may play a role in both diabetic and nondiabetic atherosclerosis, as accumulation of RAGE/AGE/S100 is seen in the vessel wall independent of diabetes, and extends the scope of RAGE blockade as a therapeutic tool for preventing vascular disease.

The effects of RAGE blockade on the proliferation and migration of SMC in the arterial wall led us to investigate other macrovascular disease processes where neointima expansion is a central mechanism; namely, restenosis induced by angioplasty (24). Evidence suggests that especially in diabetic subjects, angioplasty is associated with restenosis in 40–50% of subjects (38). One of the major underlying mechanisms of restenosis is neointimal hyperplasia resulting from proliferation of SMC (38). In a rat model of accelerated neointimal expansion, demonstrated by enhanced SMC proliferation, RAGE was seen to be up-regulated, with sRAGE reducing SMC proliferation in diabetic animals (39). In the nondiabetic state, RAGE plays a role in the underlying vascular proliferation of restenosis as seen in our studies in C57BL/6 mice (24). After denudation of the femoral artery, these mice not only displayed up-regulation of RAGE, but also AGE and S100 accumulation. Administration of sRAGE suppressed neointimal expansion in a dose-dependent manner and impacted on outcome only when treated within the first 7 days following arterial denudation (24). To further explore these findings beyond the pharmacological blockade of RAGE-ligand interaction, genetic deletion of the RAGE gene and

transgenic expression of functionally disabled RAGE in SMC was used. RAGE knockout mice were markedly resistant to neointimal expansion after arterial denudation. As the SMC was shown to be the principal RAGE-expressing cell type involved in neointimal expansion, studies employing mice transgenetically expressing the DN-RAGE form using a SMC specific promoter, also demonstrated similar resistance to vascular injury (24). The molecular mechanisms underlying this process revealed a RAGE-dependent signaling process involving Jak2 and Stat3 leading to cellular proliferation and up-regulation of tenacin-C and metalloproteinase12 (MMP12), both linked to expansion and remodeling of the extracellular matrix (24). A common criticism of animal models of restenosis is the lipid-rich, atherosclerotic-prone environment of the vessel wall seen in human subjects does not reflect that of the arteries used in rodent studies. To address this issue, we performed arterial injury in atherogenic prone apoE null mice at the age of 12 weeks, where hypercholesterolemia is present. Treatment with sRAGE from day 0 to 7 blocked the expansion of the neointima compared to the control animals treated with mouse albumin (24). These data together suggest a role for RAGE and its blockade in disease of the arteries in both diabetic and nondiabetic subjects.

Apart from macrovascular disease, diabetic vascular disease can manifest in the microvasculature affecting the eyes, kidneys, and peripheral nerves. Perhaps most important is renal disease, which occurs less frequently than retinopathy but accounts for the largest percentage of mortality of any diabetes-specific complication (40). Our initial studies of RAGE distribution by organ and cell type revealed the low-level cell-specific expression of RAGE in the podocyte of the kidney (41). However, in subjects with diabetes, RAGE was up-regulated in the glomerulus in the podocyte cells but not in endothelial or mesangial cells (41). To test the role for RAGE in activation of the podocytes and the subsequent increased excretion of serum albumin and loss of renal function, we used the insulin-resistant db/db mouse model of diabetes (42). First, up-regulation of RAGE was seen in db/db mice specifically in the podocyte by immunohistochemical staining for both RAGE and the podocyte-specific marker synatopodin (42). In addition, accumulation of CML-AGE and S100 expressing mononuclear phagocytes was detected in the db/db glomerulus. Other studies in db/db mice and in human subjects suggest a role for vascular endothelial growth factor (VEGF) as a gene up-regulated in podocytes and involved in early glomerusclerosis in diabetes (43,44). VEGF is implicated in diabetic glomerular disease by either/both the mediation of hyperpermeability and recruitment of mononuclear phagocytes (MPs) and has been also shown to be up-regulated by AGEs both *in vitro* and *in vivo* (10). Treatment with sRAGE in db/db mice not only blocked VEGF expression but also the number of infiltrated MPs in the glomerulus, a source of proinflammatory S100 proteins (42). In more long-term studies of renal disease, animals were assessed with sRAGE treatment after 27 weeks. In the sRAGE-treated group, decreased glomerular and mesangial expansion was seen in conjunction with decreased albumin excretion rate (42). These data suggest a role for RAGE in renal disease of diabetes, and we therefore investigated this further in homozygous RAGE-null mice. Diabetes was induced by streptozotocin in both wild-type and RAGE knockout mice and cell and morphological changes assessed. The increase in VEGF expression seen in the diabetic wild-type mice was blocked in the diabetic RAGE null mice, along with a lack of mesangial matrix expansion and thickening of the glomerular basement membrane (10). Studies by other groups to alter genetically RAGE expression have shown a role for RAGE in renal disease (45). Yamamoto *et al.* developed a double transgenic mouse model that developed diabetes (islet cell insulin knockout) and expresses high levels of RAGE in the vascular endothelium (45). The double transgenic animal demonstrated enhanced albuminuria, mesangial expansion, and glomerulosclerosis compared to the single transgene diabetic animal. However, in this study, significant transgenic expression of RAGE was also seen in monocytic cells, the infiltration of which accelerates glomerular disease (45). Indeed, in the study, S100 accumulation in this model released from MPs was found to be up-regulated in diabetic, but more heavily in double transgene animals, further supporting the role of AGE/S100 activation of RAGE in renal disease (45). Further studies are underway to assess further the role of RAGE/podocyte function in nephropathy.

In keeping with the inflammatory nature of RAGE in diabetic vascular disease, we investigated its role in impaired wound healing seen in diabetes. Diabetes is a major contributor to chronic wound healing, and once developed, patients become at high risk for major complications, including infection and amputation (46). To test the role of RAGE, we used the db/db mouse model of type 2 diabetes and performed full-thickness excisional wounds to generate chronic ulcers (47). Analysis of RAGE and ligand (S100 and AGE) levels in db/db wounds by histology revealed enhanced accumulation. Administration of sRAGE to block RAGE activation led to an initial accelerated inflammatory cell infiltration in wounds, over time leading to increased wound closure (47). Measurement of inflammatory cytokines revealed sRAGE treatment suppressed levels of IL-6, TNF-α, and MMP-2, 3, and 9. This eventually enhanced the generation of a thick, well-vascularized granulation tissue and increased levels of VEGF and platelet-derived growth factor-B (PDGF-B) (47).

POLYMORPHIC VARIANTS OF RAGE: THEIR ROLE IN RAGE FUNCTION AND SUSCEPTIBILITY TO VASCULAR DISEASE

The role of RAGE in vascular disease especially in diabetes highlights RAGE as a candidate gene for study to identify allelic variants and their role in RAGE pathogenesis. Epidemiological studies of patients with diabetic vascular disease suggest a strong genetic component in the development of these diseases. This is highlighted by the Diabetes Control and Complications Trial (DCCT), where siblings of subjects with diabetes demonstrate clustering of these complications and account for a 5-fold increase in incidence of nephropathy (48). These observations led to the identification and characterization of genetic variants of RAGE.

The gene for RAGE is located on chromosome 6 in the major histocompatibility complex (MHC), a region of the genome containing a number of inflammatory genes and the most dense region of genes in the genome (49,50). This region contains many overlapping genes and also genes with the same sequence encoding different genes on the opposite DNA strand (50). The RAGE gene is no exception to this, as the 5′ flanking region of RAGE, which is involved in RAGE

transcription, overlaps with the transcription factor PBX2, a gene that has a pseudogene copy on chromosome 3 (51). The RAGE gene is composed of 11 exons and a 3′UTR region (49), and within these exons a common variant in exon 3 (Gly82Ser) and 3 rare coding changes (Thr187Pro, Gly329Arg, Arg389Gln) exist (52). The Gly82Ser polymorphism has been the focus of a number of subsequent studies to assess its function and prevalence in subjects with vascular disease (Table I). This polymorphism was focused on due to its location in the ligand-binding V-domain of RAGE (52). To test the functional effects of this polymorphism *in vitro*, ligand binding assays were performed on Chinese hamster ovary (CHO) cells transfected with either the Gly82 or Ser82 and monocytes isolated from individuals from each allelic variant (53). This revealed that cells bearing the Ser82 isoform displayed not only higher ligand affinity for EN-RAGE but also, under stimulation with EN-RAGE, led to the increased activation of the proinflammatory proteins TNF-α, IL-6, and matrix metalloproteinase-9 (MMP) (53). These studies therefore suggest that the RAGE Gly82Ser polymorphism may influence inflammatory processes and hence play a role in the development of vascular disease. Studies to investigate the prevalence in human subjects initially compared the frequency of these alleles in diabetic and nondiabetic subjects with vascular disease and revealed no differences in genotype or allele frequencies (5% allele frequency for Ser82) (52). Further studies have revealed similar results with macro- and microvascular disease, but these are all limited by the population size due to the low incidence of this polymorphism (54–56).

Due to the observed up-regulation of RAGE expression in disease, genetic variation within key transcriptional regions of RAGE might impact on vascular disease. A number of studies have identified the key positive and negative regions involved in regulating RAGE transcription (57,58). Within the –1700 to +1 5′ flanking region of RAGE, a number of NF-kB sites were identified using ECV304 and human microvascular endothelial cells (HMVECs) stimulated with AGE and TNF-α (58). Screening of this region for allelic variants identified numerous polymorphisms; in particular, two common single nucleotide polymorphisms (–374 T/A and –429 T/C) and a 63-bp deletion spanning from –407 to –345 (59). Reporter gene assays to assess their function revealed a 2- (–429C), 3- (–374A), and 4- (63-bp deletion) fold increase over the wild-type sequence (59). Further investigation of transcriptional mechanisms using electrophoretic mobility shift assays (EMSAs) revealed monocytic nuclear extract

Table I. Key Allelic Variants Identified and Characterized in the RAGE Gene

RAGE allelic variant	Region of gene detected	Allele frequencies	Disease association studies	Functional implications	References
–405 to –345 deletion	5′ flanking	>99% Ins, <1% Del	No associations with ischemic heart disease in both diabetic and nondiabetic individuals.	Increased expression in reporter gene studies.	(51, 59)
–429 T/C	5′ flanking	83% –429T, 17% –429C	Association with retinopathy in diabetic individuals (24% –429C, p = 0.012).	Increased expression in reporter gene studies.	(51, 59, 60)
–374 T/A	5′ flanking	81% –374T, 19% –374A	No associations with ischemic heart disease in both diabetic and nondiabetic individuals. No association with retinopathy in diabetic individuals. Increased frequency of the –374 T allele in subjects with proteinuria and cardiovascular disease.	Increased expression in reporter gene studies. Altered binding of nuclear proteins.	(51, 59, 60)
Gly82Ser	Exon 3	95% Gly82, 5% Ser82	No association with macrovascular disease in diabetic and nondiabetic subjects. No association with diabetic microvascular disease. Increased frequency of Ser82 in diabetic skin disorders.	Increased ligand affinity and cytokine activation with Ser82 allele in macrophages.	(52–56)
1704 G/T	Intron 7	95% 1704 G, 5% 1704 T	No association with diabetic retinopathy. Association with antioxidant status in type 2 diabetic subjects.	No studies currently performed.	(74, 75)
2184 A/G	Intron 8	84% 2184 A, 16% 2184 G	No association with diabetic retinopathy. Association with antioxidant status in type 2 diabetic subjects. Association of the 2184G allele and plaque psoriasis and diabetic nephropathy.	No studies currently performed.	(74, 75)

RAGE, receptor for advanced glycation end products; Ins, Insertion; Del, Deletion.

gave a loss of a transcription factor binding site to the –374A allele (59). These data suggested these polymorphisms might affect a repressor mechanism leading to up-regulation of RAGE transcription. Initial studies of the prevalence of these polymorphisms in relation to diabetic vascular disease revealed no differences with respect to macrovascular disease, but a statistically higher prevalence of the C allele (23.6% vs. 14.9%) was seen in subjects with and without retinopathy, respectively (59). A larger recent study of 996 type 1 diabetic subjects with various degrees of nephropathy revealed a complex gene:environment interaction of the –374 A allele in renal disease (60). It was seen in patients with poor metabolic control that the –374A allele proved protective for both cardiovascular disease and lowered albumin extraction rate (60). Other studies have identified a putative polymorphism at position –1152 of RAGE associated with nephropathy in type 1 diabetic subjects (56). Subsequent studies demonstrated this is not a polymorphism but a gene:pseudogene difference between the 3′UTR of PBX2/5′ flanking region of RAGE on chromosome 6 and the PBX2 pseudogene on chromosome 3 (51). Together, these data suggest a role for polymorphisms of RAGE affecting transcription of the gene and influencing the pathogenesis of vascular disease. Further studies are required to establish the role of the promoter polymorphisms of RAGE and other polymorphic variants in other disease processes highlighted in this review.

PHARMACOLOGICAL INTERVENTION OF AGE/RAGE

Blocking the formation of AGE or interaction with RAGE are obvious targets for therapeutics. The first studies to address this were using the dicarbonyl scavenger aminoguanidine (pimagedine), which inhibits AGEs from forming by trapping them in the precursor stage (61). In animal models, aminoguanidine slowed the progression of diabetic vascular complications (62–65). Initial results from human clinical trials (Early Treatment of Diabetic Retinopathy Study) reveal an improvement in renal function; however, aminoguanidine did not impact on the progression of nephropathy (66). Other compounds have recently been used to block the effects of AGE by stopping their formation and autooxidation [pyridoxamine (67), *o*-phenylenediamine (68)] and cross-link breakers to remove already formed AGEs (ALT-711) (69). Data from animal studies reveal these to have potentially beneficial effects in the reduction and prevention of vascular disease of diabetes (69,70). Other drugs currently in use for diabetic complications have been shown to have an effect on AGE accumulation. These include the antihypertensive angiotensin-converting enzyme (ACE) inhibitor ramipril (71) and the glucose-lowering drug metformin (72), which both reduce AGE.

CONCLUSIONS

In conclusion, the biology of RAGE has extended beyond the original concept of a receptor for glycated proteins. In the animal models studied to date, blockade of RAGE using the soluble extracellular receptor region has shown a significant contribution of RAGE to vascular disease and its potential as a therapeutic tool for intervention studies in human subjects. The use of genetically manipulated animals has been a powerful tool to confirm these to be specific RAGE-dependent effects. Further studies are now underway to drive clinical translation of RAGE blockade into trials in human subjects with diabetic complications and other inflammatory disorders.

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