Glycation and diabetes: The RAGE connection

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The hyperglycaemic state seen in diabetes mellitus is associated with the development of diabetes-specific microvascular complications and accelerated macrovascular disease. Evidence implicates the formation and subsequent effects of advanced glycation end-products (AGEs) as a contributing cause. AGEs exert their effects through interaction with the Receptor for AGE (RAGE) which upregulates expression of the receptor and induces a cascade of cytotoxic pathways. Accumulation of AGE/RAGE can be seen at sites of vascular disease in both animal models of diabetes and human diabetic subjects. Blockade of RAGE in animal models of diabetes suppresses development of dysfunction in the vasculature and atherosclerosis development. Genetic studies of RAGE reveal that a number of allelic variants of RAGE occur in key protein and regulatory domains. A Gly to Ser change at position 82 and two 5′ flanking polymorphisms at position –374 and –429 lead to altered function and expression of RAGE which may impact on diabetic vascular disease development. Therapy aimed to block RAGE upregulation may prove to be useful in treating individuals with diabetic vascular disease.

DIABETES mellitus is the most prevalent metabolic syndrome world-wide with an incidence varying between 1 to 8% (refs 1, 2). Diabetes is characterized by hyperglycaemia resulting in various short-term metabolic changes in lipid and protein metabolism and long-term irreversible vascular changes. These include diabetes-specific complications of the micro-vasculature system (retinopathy, nephropathy and neuropathy) and complications of the macro-vasculature (atherosclerosis leading to heart disease, stroke and peripheral vascular disease) which are present in the non-diabetic population, but have a two to five-fold increase in diabetic subjects[3]. The incidence of diabetes is predicted to double over the next decade, possibly due to changes in lifestyles and the associated obesity in developed countries[4]. These predictions are likely to have long-term consequences on the health care delivery system, as the number of patients with type 2 diabetes is expected to continue to rise.

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Two landmark studies, the Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) have shown that strict control of hyperglycemia reduces the incidence of diabetic micro-vascular disease[5,6]. However, these studies did not show a similar consistent effect of glycemia on the impact of macrovascular disease, which suggests that factors beyond the direct effects of high glucose imparted a long-term, maladaptive 'memory' in cells of the blood vessel wall. Consequences of this phenomenon include accelerated atherosclerosis and cerebro-/cardiovascular disease, as well as enhanced neointimal expansion upon arterial injury, such as that induced by angioplasty. The identification of genes and cellular mechanisms responsible for these effects is of obvious significance and has therefore been the focus of many studies.

Various metabolic theories have been proposed to explain this relationship which include increased flux through the aldose reductase pathway, the sustained activation of protein kinase C (PKC) by increased levels of diacylglycerol (DAG), and the non-enzymatic glycation of macromolecules. The most compelling of these theories is the formation of advanced glycation end products (AGEs), evidenced by the findings of the UKPDS which indicate for every 1% increase in glycated haemoglobin levels, a 37% increase in microvascular disease was seen[6]. The formation of AGEs occurs from the reactive nature of reducing sugars (i.e. glucose) to undergo non-enzymatic rearrangements with amino groups of proteins and possibly DNA to form irreversible cross-links. Although this mechanism has only been accepted as a plausible pathway implicated in the pathogenesis of vascular disease in the last decade, the underlying biochemical reaction process has been known for almost a century.

The *in vivo* formation of non-enzymatic glycated compounds was first detected in 1969 from studies on chromotagenic mobilities of fast moving, minor hemoglobins from diabetic patients, in particular $HbA1_C$, now routinely used as a clinical tool in the management of glycaemic control in diabetic patients[7]. Its relevance was established in diabetic rats where hyperglycaemia was seen to affect the colour and protein structure changes in the lens[8]. From this hyperglycaemic-induced colour change, Cerami *et al.*[8] postulated the relevance of 'non-

enzymatic glycosylation' in the sequelae of diabetes, further evidenced by Podger *et al.*[9] who observed that cataracts with this characteristic colour change occur at an average of 10–15 years earlier in diabetic subjects.

The glycation process, otherwise known as the Maillard reaction, is divided into three key stages: the early reactions resulting in the formation of a Schiff base and Amadori products, the rearrangements of these chemical groups and the final reactions forming the classical Maillard browning products or now known as AGEs[10]. AGEs were originally shown to form over a period of weeks to months on long-lived cellular proteins, however, evidence suggests that glucose is not the only precursor of AGE, as other aldoses react more rapidly with proteins than glucose, including metabolites from the glycolysis and the polyol pathway[11]. This could, therefore, suggest a possible role for aldose reductase and this pathway in the formation of AGE. Recent findings indicate that AGE can also originate from the α-oxoaldehydes, methylglyoxal, glyoxal and the highly reactive 3-deoxyglucosone, also formed from degradation of an Amadori product[12].

A number of AGEs have been isolated or synthesized *de novo*, the two most studied being *N*-(carboxymethyl)lysine (CML)[13] and pentosidine[14]. Others include 2-2(furoyl)-4(5)-(2-furanyl)-1H-imidazole (FFI)[15], pyrraline[16], 1-alkyl-2-formyl-3,4-diglucsyl-pyrrole (AFGP)[17] and more recently 3-deoxyglucosone derived AGEs including deoxyglucasone-lysine dimer and methyl glyoxal lysine dimer[10]. Both CML and pentosidine have been found to accumulate in the skin and lens with ageing and at an accelerated rate in diabetes[18,19]. In addition, studies on pentosidine identified increased levels in the skin of diabetics, associated with the severity of the complications found in these patients[20] and increased levels in patients with microalbuminuria[21].

The role of AGE in vascular disease was first identified by their ability to cross-link proteins of the vascular wall leading to the thickening of vessels and leakage from the vasculature[22]. Further evidence to support this was seen in animal models of diabetes which demonstrated that the inhibitor of AGE formation, aminoguanidine, blocked the cellular mechanisms underlying both micro and macrovascular disease[22]. AGE have been shown to produce a variety of toxic effects by a number of mechanisms. Firstly, the formation of AGE occurs on the extracellular matrix leading to the trapping of proteins and eventual narrowing of the lumen. Secondly, AGE formation occurs intracellularly through rapid intermediates of glucose metabolism, altering protein structure and function. Thirdly, AGE interact with AGE binding receptors which remove and degrade AGEs and activate proinflammatory and prothrombotic pathways. A variety of candidates receptors have been identified which include the AGE–receptor complex (AGE-R1-3)[23,24], macrophage scavenger receptors (type I and II)[25,26], the receptor for advanced glycation end products (RAGE)[27], CD-36 (ref. 28) and most recently LOX-1[29]. To-date the majority of studies support RAGE as a central role in the biology and pathogenesis of AGEs.

RAGE was initially identified by its ability to bind and internalize AGEs, however, subsequent studies now suggest that RAGE is a signalling receptor, as ligand engagement modulates cellular function to show RAGE is not likely to be a scavenger of AGEs[30]. Characterization of RAGE identified it as a member of the immunoglobulin superfamily of receptors[31]. RAGE is composed of three immunoglobulin domains, one V-type and two C-type domains, with a single transmembrane region and a short high charged cytosolic tail of 43 amino acids necessary for signalling[32]. Indeed, studies have suggested that transient transfection of (endogenous) RAGE-bearing cells with RAGE constructs in which solely the cytosolic domain was deleted revealed that upon ligand stimulation, cellular signalling and altered gene expression were effectively suppressed[33]. These observations suggested that deletion of the RAGE cytosolic domain imparted a 'dominant negative' (DN) effect. We have confirmed these observations in both *in vitro* and *in vivo* analyses; in the latter employing transgenic animals bearing DN-RAGE[34].

The AGE-binding domain of RAGE was experimentally determined to be within the first 30 amino acids of the V-domain[35]. Two isoforms of RAGE were originally detected consisting of 45 kDa and 35 kDa (ref. 27). This was later confirmed to occur in most cells studied, except for neuronal tissue where a 48 kDa and 23 kDa were isolated, and suggested to arise from posttranslational processing of RAGE[36]. The 35 kDa isoform of RAGE was experimentally produced by producing a truncated version of RAGE including the extracellular domain of RAGE, and designated as soluble RAGE (sRAGE)[37]. RAGE is expressed by most cell types including vascular endothelium and smooth muscle, monocytes, macrophages, glomerular epithelial cells and neuronal cells[36].

RAGE is normally expressed at low levels in most tissues except lung, however, in atherosclerotic lesions from diabetic subjects RAGE is highly upregulated compared to healthy subjects[36]. This is also seen in animal models of diabetes which show up-regulated RAGE in vascular endothelium and smooth muscle[36,38]. The increased expression of RAGE has been demonstrated in a variety of other diabetic vascular disease states, including the vascular smooth muscle[39], the glomeruli, podocytes and tubular epithelial of the kidney[40], the endothelium in peripheral occlusive vascular disease[41] and the fibrovascular membranes of the retina[42]. However, RAGE expression is increased in a number of disease states, involving non-AGE related inflammatory diseases[40].

AGE and RAGE were found to co-accumulate with diabetes in renal glomerulus, retina and aorta, and both AGE levels and RAGE expression was reduced by treatment with aminoguanidine[43]. The accumulation of AGE

and RAGE was studied in more depth in cardiovascular tissue of the diabetic rat, demonstrating the increased accumulation of AGE with diabetes and increased expression of RAGE in the endothelium and vascular smooth muscle with duration of hyperglycaemia[38].

Investigations into the molecular mechanism underlying RAGE activation in these processes, revealed the induction of an oxidative process arising from the infusion of AGE into rats, dependent on RAGE[44]. An AGE/RAGE mechanism was shown to induce dysfunction in the endothelium resulting in hyperpermeability, reversed by the blockage of AGE/RAGE interaction by antibodies to RAGE[45]. At the cellular level, AGE/RAGE was found to induce the activation and nuclear translocation of Nuclear Factor-κB (NF-κB), a transcription factor responsible for the induction of endothelium/leukocyte adhesion molecules implicated in the initiation of the atherosclerotic lesions and other vascular disorders[46]. Expression of RAGE was found to be dependent on a number of key NF-κB sites[47], which demonstrated a possible feedback-loop of increased expression of RAGE arising from AGE/RAGE interaction.

The involvement of NF-κB via RAGE and its role in the pathogenesis of vascular disease was further revealed by the finding of increased expression of VCAM-1 in the endothelium resulting from exposure to AGE[48]. VCAM-1 has been shown to be expressed on the endothelium in atherosclerotic plaques, resulting in an increase in monocyte adhesion[49]. Monocytes bearing RAGE at their surface are recruited by a chemotaxic mechanism to sites of AGE accumulation[50], whereupon they infiltrate to the subendothelium to eventually form foam cells, an early step in the development of atherosclerosis[51]. In addition to this, the secretion of chemoattractant molecules from vascular smooth muscle cells (VSMC) could be induced by AGE/RAGE, demonstrating a further mechanism to induce monocyte infiltration[52].

This mechanism of chemotaxis could also be demonstrated using AGEs isolated directly from diabetic patients, showing RAGE to bind true physiological AGEs[48]. For all other identified AGE-receptors, experiments have only been performed using *in vitro*-produced AGEs, which are proposed to be highly over-glycated in comparison with *in vivo* occurring AGEs[53]. This is thought to result in a high degree of non-specific binding to the cell surface, which can render the non-specific AGE-binding effect[54].

The interaction of AGE with the endothelium also leads to the synthesis of a number of other proteins, with relevance to both macro and micro vascular complications. The increased expression of tissue factor (TF) by both endothelium[55] and macrophages[56] occurs via an AGE/RAGE mechanism, which may initiate a procoagulant state, further enhanced by the AGE/RAGE induction of Plasminogen Activator Inhibitor-1 (PAI-1), an attenuating serine protease of fibrinolysis[57]. Furthermore, the AGE/RAGE interaction induces endothelin-1 expression, a potent vasoconstrictor[58] and consequently inhibits the production of prostacyclin and nitric oxide, a potentially contributory factor to hypertension in diabetes[59]. The inhibition of prostacyclin production has consequences in the initiation of retinopathy, as a RAGE-dependent mechanism can be shown to reduce prostacyclin production by the endothelium[60] and result in the loss of pericytes[61], the earliest visible marker of diabetic retinopathy[61,62].

These AGE/RAGE effects were demonstrated to be reversible at the cellular level by blocking their interaction using either antibodies directed against RAGE or using sRAGE, suggesting a possible therapeutic value in vascular disease[63]. Previous studies had demonstrated dysfunction of the endothelium to occur via an AGE/RAGE mechanism by infusing red blood cells (RBCs) from diabetic rats into normal animals. This resulted in increased RBC adhesion to the endothelium, the induction of oxidative stress and general dysfunction of the endothelium[64]. Renard *et al.*[65] repeated these studies and injected sRAGE into the rats, which fully reversed the hyperpermeability resulting from the AGE-RBCs binding to the endothelium. The beneficial effects of sRAGE in the full development of vascular disease was demonstrated in diabetes-induced streptozotocin mice, genetically engineered to rapidly develop atherosclerosis (apoE-null)[66]. Infusion of mice with sRAGE suppressed development of accelerated atherosclerotic lesions in a glycaemic and lipid-independent manner[66].

The biology of RAGE extends beyond the scope of binding and mediating the effects of AGEs. The finding that RAGE was upregulated in non-diabetic subjects with vascular disease and in non-diabetic Apo-E mice suggest the role of other non-AGE ligand activating RAGE[41]. Studies have so far identified RAGE to bind a variety of ligands which include the neural development regulator, amphoterin[67] and the protein involved in Alzheimer's disease, β-amyloid[68] (see Table 1). The role of the high

Table 1. Known ligands for RAGE and their potential impact in homeostasis and pathophysiological settings

Ligands for receptor for AGE	Physiologic/pathophysiologic impact
Advanced glycation endproducts	Diabetes, renal failure, amyloidoses, (e.g. CML-adducts) inflammation, oxidant stress, aging
Amyloid-βpeptide and β-sheet fibrils	Alzheimer's disease, amyloidoses
S100/calgranulins	Development, neurite outgrowth inflammation, tumour biology
Amphoterin	Development, neurite outgrowth inflammation, tumour biology

expression pattern of RAGE seen in lung and in non-hyperglycaemic disease states may be explained by the recent isolation of an endogenous ligand for RAGE termed Extracellular Newly identified RAGE-binding protein or EN-RAGE[33]. EN-RAGE, a 12 kDa protein was found to bind and activate RAGE on endothelial and monocytic cells[33]. By sequence homology, EN-RAGE was identified as S100A12, a member of the S100 family of EF left hand calcium-binding proteins[33]. Although these proteins consist of ≈ 19 structurally similar members, they have a variety of distinctly different intracellular and extracellular roles[69]. The S100 genes are located in a cluster on chromsome 1q21 and also identical in their genomic organization, comprising of 3 exons coding for the 5′ untranslated region, N-terminal EF-hand, and carboxyl terminal EF-hand respectively[69]. EN-RAGE is expressed by granulocytes and monocytes[70], its original proposed role to act as a proinflammatory mediator by recruiting monocytes[71]. Interaction with RAGE on mononuclear phagocytes, endothelial cells and lymphocytes results in increased expression of proinflammatory mediators including tumour necrosis factor-α (TNF-α), VCAM-1 and interleukin-1/2 (IL-1/2)[33]. Studies of EN-RAGE/RAGE in vascular disease have identified EN-RAGE upregulation in atherosclerotic plaques together with increased RAGE and AGEs[72].

These findings suggest RAGE to be a key target for both drug intervention studies and as a candidate for genetic studies. A role for genetic susceptibility in the development of diabetic vascular disease is supported by family studies of clustering of retinopathy and nephropathy. In a follow-up study to address these issues the DCCT investigated the type, presence and severity of complications in 241 first-degree relatives of the previously investigated group, with either type 1 or 2 diabetes[73]. With nephropathy, it appeared that a familial factor accounted for the five-fold increase in relatives of DCCT subjects and with retinopathy a clustering was seen with severity[73]. These observations led to studies to investigate the presence of allelic variation in the RAGE gene and their effects on expression and function.

The gene for RAGE is located on chromosome 6p21.3 in the major histocompatibility complex (MHC)[31], in perhaps the most gene-rich area of the genome containing an average of one gene per 10 kb of DNA and many overlapping genes[74]. The gene for RAGE is no exception to this, as the 5′ flanking region from –505 in the 5′ direction overlaps with PBX2, a gene which has a non-functional pseudogene copy on chromosome 3 (ref. 75). The RAGE gene was assessed for the presence of novel polymorphisms within exons and gene regulatory regions which might affect function and expression of RAGE[76–79]. At first the 11 exons and 3′ UTR of RAGE were identified and screened and a number of amino acid changes which included a common variant in exon 3 (Gly82Ser) and 3 rare coding changes (Thr187Pro, Gly329Arg, Arg389Gln) were identified[76]. Additional studies have identified a number of other polymorphisms in the coding region of RAGE (summarized in Table 2). However, these have all proven to be either non-codon changes or rare amino acid changing variants[78,79].

Further studies were performed on the common Gly82Ser polymorphism as it was found to occur within the ligand-binding domain of RAGE, making this a potentially important gene variation. The Gly82 and Ser82 isoforms were expressed in cells and the consequences of the Gly82Ser on receptor function investigated. In Chinese Hamster Ovary cells (CHOs) and macrophages isolated from human subjects, the Ser82 isoform displayed a higher affinity for the EN-RAGE ligand, and under stimulation with EN-RAGE led to the increased activation of the proinflammatory proteins, TNF-α, IL-6 and matrix metalloproteinase (MMP)[80]. These findings suggest the possibility that the Ser82 allele may play an important role in the inflammatory component of vascular disease. Indeed, a number of studies have been performed to assess the prevalence of the Gly82Ser polymorphism in vascular disease of both diabetics and non-diabetics. Initial studies in diabetic subjects identified no differences in genotype or allele frequencies between subjects with or without (5% allele frequency for Ser82) macrovascular disease[76]. This result has been seen in other studies, in diabetics with macrovascular[81] and microvascular[79,82,83] disease, but all of these studies have been limited in the size of the populations and the low frequency of the Ser82 allele. However, the functional data seen *in vitro* with the Gly82Ser polymorphism, supports a role for the Ser82 allele in heightening the inflammatory responses in vascular disease. This view may be supported by the identification of increased Ser82 distribution in diabetic subjects with microvascular dermatoses[78], psoriasis vulgaris[84] and in subjects with rheumatoid arthritis[80]. Both this result and the lack of association of Gly82Ser with vascular disease in diabetes need to be established in larger patient groups. The fact that polymorphisms of the RAGE coding region have been either very rare coding changes or the infrequent Gly82Ser polymorphism suggests the role for other regions of the RAGE gene affecting outcome of the polygenic nature of microvascular and macrovascular disease. Studies to address these issues investigated other key regions of RAGE, specifically the transcriptional regulatory regions of RAGE potentially affecting RAGE expression[77,79].

Characterization of the RAGE 5′ regulatory region identified major sites of positive and negative regulation within the –1700 to +1 5′ flanking region of RAGE using deletion reporter gene constructs[47,85]. Using DNase I footprinting and mutagenesis, a number of NF-κB sites were identified to exist in the –1543 to –738 and –738 to –587 regions[47], confirmed using ECV304 and human microvascular endothelial cells (HMVECs) stimulated with AGE and TNF-α[85]. Subsequently, a number of

Table 2. Polymorphisms of the RAGE gene

RAGE polymorphism	Region detected	Allele frequencies	Disease association studies	Functional implications	References
–1420 (GTT)*n*	5′ Flanking	N/A			77
–1393 G/T	5′ Flanking	N/A			77
–1390 G/T	5′ Flanking	N/A			77
–1202 G/A	5′ Flanking	N/A			77
–405 to –345 deletion	5′ Flanking	> 99% Ins, < 1% Del		Increased expression in reporter gene studies Increased expression in reporter gene studies	77
–429 T/C	5′ Flanking	83% –429T, 17% –429C	No associations with ischaemic heart disease in both diabetic and non-diabetic individuals. Association with retinopathy in diabetic individuals (24% –429C, $P = 0.012$)	Increased expression in reporter gene studies	77, 86
–374 T/A	5′ Flanking	81% –374T, 19% –374A	No associations with ischaemic heart disease in both diabetic and non-diabetic individuals. No association with retinopathy in diabetic individuals. Increased frequency in non-small cell lung cancer (39% –374A, $P < 0.05$)	Increased expression in reporter gene studies. Altered binding of nuclear proteins	77, 79, 86, 87
Ala2Ala (GCT/GCA)	Exon 1	86% T, 14% A			76
67 C/G	Intron 1	83% C, 17% G			76
Lys37Ser	Exon 2	> 99% Lys37, < 1% Ser37			78
Arg77Cys	Exon 3	> 99% Arg77, < 1% Cys77			78
Gly82Ser	Exon 3	95% Gly82, 5% Ser82	No association with macrovascular disease in diabetic and non-diabetic 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	76, 78–82 84
Val89Val (GTG/GTC)	Exon 3	95% G, 5% C			78
Gly90Gly (GCT/GCA)	Exon 3	95% T, 5% A			76
718 G/T	Intron 3	92% 718 G, 8% 718 T			78
Thr187Pro	Exon 6	> 99% Thr187, < 1% Pro187			76
1704 G/T	Intron 7	95% 1704 G, 5% 1704 T	No association with diabetic retinopathy. Association with anti-oxidant status in type 2 diabetic subjects		78, 88
A insertion 1727	Intron 7	N/A			78
His305Gln	Exon 8	> 99% His305, < 1% Gln305			78
Ser307Cys	Exon 8	> 99% Ser307, 1% Cys307			78
Gly329Arg	Exon 8	> 99% Gly329, < 1% Arg329			76
2117 A/G	Intron 8	N/A			78
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		78, 88–89
2224 A/G	Intron 8	N/A			78
2245 G/A	Intron 8	92% 2245 G, 8% 2245 T	No association with diabetic retinopathy		78
2249 A/G	Intron 8	N/A			78
2741 G/A	Intron 9	N/A			78
Leu363Leu (CTG/TTG)	Exon 10	99% C, 1% T			76
Arg389Gln	Exon 10	> 99% Arg389, < 1% Gln389			76
CA deletion 3089	3′ UTR	< 1% CA deleted			78

polymorphisms within the RAGE 5′ flanking region were identified (see Table 2) which included two common single nucleotide polymorphisms (–374 T/A and –429 T/C) and a 63 bp deletion spanning from –407 to –345, all within a region of negative gene regulation[77]. Functional analysis of these polymorphisms by reporter gene assays in HepG2 cells gave a 2 (–429C), 3 (–374A) and 4 (63 bp deletion) fold increase in chloroamphenicol acetyl transferase (CAT) expression relative to the wild type construct[77]. These were studied further by investigating the influence of –429 T/C and –374 T/A on U937 and HepG2 transcription factor binding using electrophoretic mobility shift assays (EMSAs). The –374A allele caused a complete loss of a DNA : protein complex from both cell lines[77]. Together with the reporter gene assays this suggests that this polymorphism affected a repressor binding to RAGE leading to up-regulation. The prevalence of these polymorphisms in diabetic subjects revealed no difference in genotype or allele frequency with either polymorphism in subjects with (–429T 81%, –429C 19%; –374T 83%, –374A 17%) or without macrovascular disease (–429T 83%, –429C 17%; –374T 81%, –374A 19%)[86]. There was, however, a significant association seen between the –429 T/C polymorphism and retinopathy[77]. Diabetic subjects with retinopathy were found to have a statistically higher prevalence of the C allele 23.6% vs 14.9%, with vs without retinopathy)[77]. Other investigators additionally identified a C to A polymorphism in the RAGE 5′ flanking region at –1152 (–1139 from actual +1 site) which was found to associate with nephropathy in type 1 diabetic subjects[79]. However, this was later shown to not be a polymorphism, but to be in fact 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 (ref. 75). Again, larger study numbers are required to verify the results seen with the –374 T/A and –429 T/C polymorphisms and to establish whether these gene variants have a causative role in the pathogenesis of vascular disease.

In conclusion, it is highly likely that glycation and the ultimate formation of AGEs are central to the pathogenesis of diabetic vascular disease. From *in vitro* and *in vivo* studies, the interaction with their receptor, RAGE presents a novel target for drug intervention to reduce and prevent the development of the debilitating side effects of hyperglycaemia. Taken together with genetic susceptibility data from RAGE allelic variants which may influence the disease progression further, it may be possible to tailor individual therapeutics against RAGE to ameliorate disease progression.

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