

# Interactions Between Advanced Glycation End-Products (AGE) and their Receptors in the Development and Progression of Diabetic Nephropathy – are these Receptors Valid Therapeutic Targets

Karly C. Sourris<sup>1,\*</sup> and Josephine M. Forbes<sup>1,2</sup>

<sup>1</sup>JDRF Albert Einstein Centre for Diabetes Complications, Glycation and Diabetes, Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia and <sup>2</sup> Department of Immunology, Monash University, AMREP precinct, Melbourne, Victoria Australia

**Abstract:** Diabetes, is a metabolic disorder characterised by chronic hyperglycaemia, hypertension, dyslipidaemia, microalbuminuria and inflammation. Moreover, there are a number of complications associated with this condition including retinopathy, neuropathy and nephropathy. Diabetic nephropathy, is the major cause of end-stage renal disease in Western societies affecting a substantial proportion (25-40%) of patients with diabetes. Advanced glycation end products (AGEs) have been identified as important modulators of the development and progression of diabetic nephropathy, through both receptor dependant and independent interactions. AGEs elicit their receptor mediated effects *via* their engagement with numerous receptors and binding proteins which are broadly thought to be either inflammatory (RAGE and AGE-R2) or clearance receptors (AGE-R1, AGE-R3, CD36, Scr-II, FEEL-1 and FEEL-2). Modulation of AGE receptor expression is an important potential therapeutic approach worth consideration as a treatment for diabetic nephropathy and likely applicable to other vascular complications.

**Key Words:** Advanced glycation end-products (AGEs), diabetic nephropathy, modulation of AGE-receptors, RAGE, AGE-R1, soluble-RAGE.

## INTRODUCTION

Today, it is estimated that approximately 180 million individuals worldwide have diabetes and WHO predicts that this is likely to double by the year 2030. The incidence of diabetes is increasing across the board, irrespective of age, sex or ethnicity [1]. Diabetes is a metabolic disorder characterised by chronic hyperglycaemia, and often co-morbidities such as hypertension, dyslipidaemia and inflammation, with the major types being type 1 (insulin-dependant) or type 2 (commonly non-insulin dependant) [1, 2]. Both forms of diabetes are associated with micro- and macrovascular complications which include retinopathy, neuropathy, cardio/cerebrovascular disease and nephropathy which are the major cause of mortality and morbidity in diabetic patients [3, 4]. Indeed, diabetic nephropathy (DN), is the major cause of end-stage renal disease in Western societies affecting a substantial proportion of patients with type 1 (25-40%) and type 2 (30-40%) diabetes [5, 6]. This disease is characterised by morphological, biochemical and functional alterations within the kidney [7-11]. In this review we discuss the importance of AGE modifications as ligands for AGE receptors and the relevance of this interaction to the development of diabetic renal disease. In addition, we investigate the therapeutic benefits of manipulation of AGE-receptor expression and interactions as a therapeutic target for the treatment of DN.

## DIABETIC NEPHROPATHY

Diabetic nephropathy is defined as a progressive decline in glomerular filtration rate, accompanied by proteinuria and other end-organ complications such as retinopathy [2]. Diabetic nephropathy progresses to end-stage renal disease *via* a number of stages including, normoalbuminuria, microalbuminuria/incipient diabetic nephropathy, macroalbuminuria and finally end-stage renal disease [2, 4]. Indeed, progression to end stage renal disease is enhanced by hyperglycaemia, hypertension and proteinuria, which are all common in diabetes [3, 12-14].

Renal disease in diabetic patients is characterised by haemodynamic (hyperfiltration and hyperperfusion) as well as structural abnormalities (glomerulosclerosis, alterations in tubulointerstitium including interstitial fibrosis) and metabolic changes [15]. Within glomeruli, there is thickening of basement membranes, mesangial expansion and hypertrophy and glomerular epithelial cell (podocyte) loss [16]. In conjunction, disease progression is also seen in the tubulointerstitial compartment causing expansion of tubular basement membranes, tubular atrophy, interstitial fibrosis and arteriosclerosis [12]. To date, the most effective clinical treatments to prevent the progression of diabetic nephropathy are anti-hypertensives, which target the renin-angiotensin system [17-19].

## ADVANCED GLYCATION END-PRODUCTS

AGEs are a heterogenous and complex group of compounds, which play an important role in the development of diabetic nephropathy. They often present as a yellow-brown

\*Address correspondence to this author at the Baker IDI Heart and Diabetes Institute, PO Box 6492, St Kilda Rd Central, Melbourne, 8008, Australia; Tel: +61 3 8532 1124; Fax: +61 3 8532 1111; E-mail: Karly.Sourris@bakeridi.edu.au

pigmentation, may be fluorescent and a number are primarily cross-links between proteins [20-22]. AGEs are formed as a result of non-enzymatic biochemical reactions initiated as the Maillard Reaction. The Maillard reaction was described in 1912 following the first documented observation of the browning of sugars when combined with amino acids [23]. This reaction is a multi-step process, where a reactive carbonyl, from glucose or its derivatives are attached to lysine and arginine residues on proteins, amino acids and nucleic acids [24, 25]. Following further condensation, rearrangement and other reactions, the intermediate compounds of which some are "Schiff" bases and amadori products, are further irreversibly modified to become advanced glycation end products (AGEs) [9, 25]. Physiologically, advanced glycation is thought to play an important role in the identification of senescent molecules, which are then subsequently cleaved and cleared, primarily *via* the kidneys [26].

Within the body, AGEs accumulate from both endogenous and exogenous sources. Intracellularly, AGEs are formed as a by-product of a number of important biochemical reactions including auto-oxidation of glucose to glyoxal, decomposition of amadori products and the fragmentation of glyceraldehyde. The reactive intracellular intermediates formed during these reactions, such as methylglyoxal, react with the amino groups of both intracellular and extracellular proteins to form AGEs [21, 27].

AGEs may be categorised on the basis of their action and function as either non-cross-linking adducts and cross-linking adducts such as hydroimidazoles [16]. Some of the best characterised AGEs to date, include N-carboxymethyllysine (CML), N-carboxyethyllysine (CEL), pentosidine, imidazole, glyoxallysine dimer (GOLD) and pyrroline [9, 14, 16, 21]. In addition, there are a number of exogenous sources of AGEs identified in recent times, including food and tobacco smoke, which also contribute to the body's AGE pool [28, 29].

Under normal physiological conditions, AGEs are cleared from the body *via* the kidney, following their degradation by reductase enzymes within cells such as macrophages. The kidney *via* a multi-step process, filters AGE modified-peptides and proteins. Following filtration *via* the glomeruli, they are subsequently reabsorbed by the proximal tubules where they are often further degraded and then excreted into the urine [20]. In homeostasis, the rate of renal AGE removal is proportional to creatinine clearance, ensuring that there is no excess accumulation of tissue AGEs.

In metabolic disorders, such as diabetes, there is a marked increase in a number of factors which promote the formation and accumulation of AGEs within various susceptible organs, in particular, the kidney. As a direct result of the hyperglycaemia characteristic of diabetes, there is marked increase in both carbonyl and oxidative stress, which each promote *in vivo* AGE accumulation [30, 31].

Excessive AGE accumulation can elicit a variety of deleterious effects on tissues and organs. These include altering the structure and function of both intracellular and extracellular molecules, increasing oxidative stress, modulation of cell activation, enhancement of signal transduction pathways and increasing the activation and expression of cytokines and

growth factors. These actions have been shown to be mediated *via* both receptor dependant and independent mechanisms [9, 22].

## AGE RECEPTORS

The receptors for AGE are important modulators of the deleterious effects of these compounds. Receptors for AGEs may be loosely grouped as either inflammatory (RAGE, AGE-R2) or clearance type receptors (AGE-R1, AGE-R3, CD36, Scr-II, FEEL-1 and FEEL-2) [9, 32-36]. Vascular, renal, neuronal and haematopoietic cells are all known to express receptors for AGEs [37] Fig. (1).

## RECEPTOR FOR ADVANCED-GLYCATION END-PRODUCTS (RAGE)

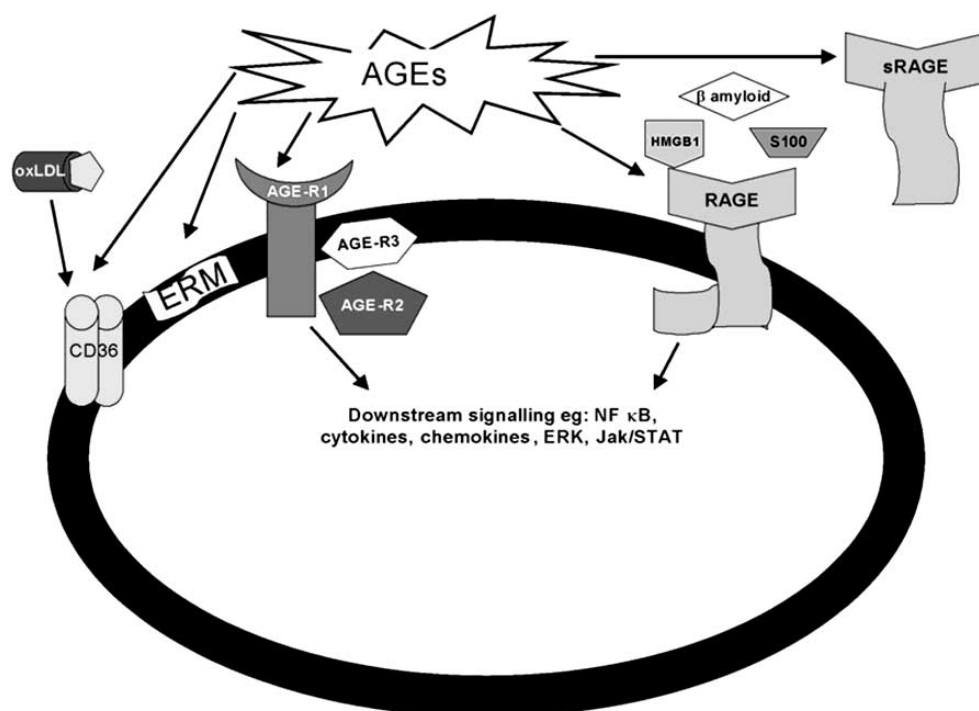
One of the best characterised receptors for AGEs is the receptor for AGE (RAGE). RAGE (45kD) is a member of the immunoglobulin superfamily, expressed on the surface of monocytes, macrophages, neurons, proximal tubular cells [38] and glomerular epithelial cells (podocytes) [39], mesangial, endothelial, smooth muscle and fibroblast cells [40-42]. RAGE is a multifunctional receptor as it is capable of binding to a number of ligands other than AGE modifications including amyloid- $\beta$ -peptides, amyloid A, s100 calgranulins and amphoterin (HMGB1) [41, 43, 44] Fig. (1). Its major physiological role is thought to be in host-pathogen defence.

The RAGE gene is located on chromosome 6 adjacent to the HLA locus in both human and mouse [40, 41] and its transcription is known to be both constitutive and inducible. RAGE is expressed during embryogenesis whilst it is down-regulated in adult life during homeostasis in most tissues [41].

The RAGE protein consists of three immunoglobulin-like regions, one v-domain and two c-domains, in addition to transmembrane and cytoplasmic regions [45-48] Fig. (1). There are a number of identified isoforms of RAGE, which lack either the cytoplasmic or extracellular domains. These include soluble RAGE (sRAGE), thought to be the result of proteolytic cleavage of RAGE from the cell surface [49] Fig. (1). Soluble RAGE binds AGEs with a high affinity and thought of as a possible scavenger receptor for AGEs [50]. Endothelial cells are known to secrete an isoform of RAGE (es-RAGE), which is c-terminal splice variant of RAGE, which lacks a trans-membrane and effector domain and may bind to extracellular ligands independently of cell contact. Dominant-negative (DN-RAGE) is a recombinant isoform, which may bind to ligands and suppresses the intracellular signalling of full-length RAGE. Finally, NT-RAGE, lacks an amino terminus however its function is still unclear [16, 41, 51].

## AGE-CLEARANCE RECEPTOR COMPLEX (AGER1, AGE-2 AND AGE-R3)

The AGE-receptor complex is thought to consist of AGE-R1, AGE-R2 and AGE-R3 and fundamental in the clearance of AGEs. The interaction amongst these receptors is thought to drive the degradation of AGE modified molecules into smaller fragments for clearance by the kidney.



**Fig. (1).** Schematic representation of cellular and soluble AGE-receptors identified and the signalling pathways which they transduce.

AGE-R1 (OST-48, 48kD), as a member of the oligosaccharyl-transferase protein family, is a type 1 integral membrane protein which has a short extracellular amino terminal, a single transmembrane region and cytoplasmic c-terminal domain [52, 53]. AGE-R1 was the first AGE-receptor cell surface clearance receptor identified and is anchored within the endoplasmic reticulum where it is thought to be a stabilising molecule for the oligosaccharyltransferase (OST) complex and thus referred to as OST-48 [52, 53] Fig. (1).

AGE-R2 (80K-H, 90kD), is a tyrosine phosphorylated protein which is located within the plasma membrane Fig. (1). This receptor was initially thought to act as a substrate for protein kinase C, however it has been recently shown to be involved in the intracellular signalling of several receptors including fibroblast growth factor (FGF)-receptor [9, 52-56]. Whilst it does not directly bind to AGEs, it is highly efficient in phosphorylation of AGE receptors, thought to be contributive to the early stages of AGE signal transduction [52, 55].

AGE-R3 (galectin-3, 32kD) is expressed in both the cytoplasm and the nucleus in addition to the cell surface of macrophages, eosinophils, mast cells, epithelium of gastrointestinal and respiratory tract, sensory neurons and renal cells [57, 58] Fig. (1). AGE-R3 has been shown to bind to carbohydrates, laminin and IgE molecules. Furthermore, it has a number of cellular functions including apoptosis, activation, inflammation and tumour growth [53, 58-62]. The inflammatory role of galectin-3 has been extensively investigated. In particular, it has been shown to be a potent inducer of matrix metalloproteinase-3 and extracellular matrix protease, ADAMTS-5 which are known to break down cartilage and extracellular matrix [63]. Moreover, it exhibits immunoregulatory potential to attract eosinophils when expressed in T-lymphocytes [64]. In pancreatic, endothelial and melanoma

cancer cells, increased expression of galectin-3 drives their proliferation and survival, demonstrating its anti-apoptotic nature. [65-67].

### SCAVENGER RECEPTORS (CD36, FEEL-1 AND FEEL-2)

CD36 is a highly glycosylated 88kDa protein which binds various molecules including fatty acids, collagen and oxidised LDL (OxLDL) [68, 69] Fig. (1). It is expressed on the cell surface of both adipocytes and macrophages [70, 71]. The major pathophysiological functions of CD36 include scavenging oxLDL in macrophages and fatty acid transport in a number of cell type including adipocytes [71]. CD36 binds AGEs with a high affinity leading to subsequent receptor mediated endocytosis [72-74].

Fascilin, EGF-like, laminin-type EGF-like and link domain-containing scavenger receptors-1 and 2 (FEEL-1 and FEEL-2) bind AGE modified proteins. Moreover, as with other AGE receptors, FEEL-1 and FEEL-2 are multiligand receptors which are known to endocytose bacteria, modified LDL, as well as AGEs [75]. These receptors differ in their structure to other scavenger receptors. FEEL-1 protein is 2570 amino acids in length. There is approximately 40% amino acid homology shared between FEEL-1 and FEEL-2. At the mRNA level, both receptors have been detected in spleen and lymph nodes, however, cell surface expression has only been identified for FEEL-1 on CD14-positive mononuclear cells [71, 76].

### ERM FAMILY OF AGE BINDING PROTEINS

The ezrin, radixin and moesin (ERM) proteins are known to link the cytoskeleton of cell to the plasma membrane and

are thought to play a role in cellular shape, change, motility and adhesion [77] Fig. (1). This group of proteins have been recently identified as novel binding proteins for AGEs in kidney of diabetic rats [78]. The interaction between ERM and AGEs may also contribute to the development of diabetic nephropathy [78].

### AGES AND DIABETIC NEPHROPATHY

AGEs contribute to the pathogenesis of diabetic nephropathy *via* receptor mediated mechanisms and indirectly *via* the generation of reactive oxygen species and by altering extracellular matrix (ECM) integrity. Circulating levels of AGEs in diabetic patients are elevated with decreased renal function [79]. Furthermore, AGE accumulation in tissues correlate with the severity of organ injury, particularly within glomerular lesions [80, 81]. Early in the development of diabetic nephropathy, excesses of AGEs such as pentosidine and CML, have been identified in the expanded mesangial area and thickened glomerular capillary wall [82, 83]. Indeed the accumulation of skin collagen associated AGEs is predictive of the onset of diabetic renal disease in type 1 diabetic patients [84]. *In vitro*, AGEs have been shown to increase transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) and ECM expression in glomerular endothelial and mesangial cells which is enhanced in hyperglycaemic conditions [85-88].

Dietary AGEs, are also thought to contribute to the development of diabetic nephropathy. Diets low in AGE content, when fed to non-obese diabetic mice (type 1) and db/db mice (type 2) reduced glomerular lesions, creatinine/albumin ratios and renal TGF $\beta$ 1 expression when compared to their high AGE counterparts [89]. Moreover, diets high in AGE content are known to impair insulin sensitivity further confounding downstream complications [90]. Various agents, including LR-90 [91], aminoguanidine [92], and ALT-711 [93] are potent in reducing AGE accumulation in renal tissues in experimental diabetic nephropathy, subsequently improving renal function. Many other agents have elicited similar benefits and have been extensively reviewed previously [94-96]. These benefits are also seen in the clinical context, with agents such as metformin, which decrease toxic dicarbonyls and AGEs in addition to its anti-hyperglycaemic effects [97]. In addition, pyridoxamine, an intermediate of vitamin B<sub>6</sub>, attenuates the progression of human diabetic nephropathy [98]. Furthermore, benfotiamine (liposoluble vitamin B1 derivative), decreases AGE accumulation, inflammation and improves vascular function in type 2 diabetic patients consuming diets high in AGE content [99].

### ALTERED AGE-RECEPTOR EXPRESSION IN DIABETIC NEPHROPATHY

Diabetes alters the expression of a number of AGE-receptors thought to drive the development and progression of diabetic nephropathy, in particular, the expression of RAGE on cells such as podocytes and tubular epithelial cells [100-103]. Double transgenic mice which over-expressed inducible nitric oxide synthase (iNOS) and human RAGE in their vasculature, exhibited significant increases in kidney weight, albuminuria, glomerulosclerosis, and increases in serum creatinine relative to their control counterparts [104].

Interestingly, these deleterious changes observed in the double transgenic mice were ameliorated when treated with the thiazolium derivative, OPB-9195, which is thought to reduce AGE accumulation by trapping reactive carbonyls [104]. This suggests that it is RAGE-ligand interactions, which are responsible for the renal disease observed within this model. Yamamoto and colleagues subsequently developed a triple transgenic model, which overexpressed megasin in addition to iNOS and RAGE [105]. These mice had an earlier onset of diabetic nephropathy [105]. To further elucidate the importance of RAGE as a modulator of diabetic nephropathy, a number of RAGE-deficient models have been established [106]. Indeed, RAGE deletion confers renoprotection in rodent models of diabetic nephropathy [39, 101, 106]. Since physiologically, RAGE is directly involved in pathogen defence and innate immunity, RAGE KO mice are also resistant to septicemia, which is ameliorated following reinstatement of vascular RAGE expression [107].

Another AGE receptor postulated to be involved in the development of diabetic nephropathy is AGE-R1, although converse to RAGE this is likely *via* a decrease in expression. In an experimental model of type 1 diabetes, renal AGE-R1 expression is reduced in association with a concurrent increase in AGE deposition and progression to diabetic nephropathy [53, 108]. C57BL/6 mice when fed diets abundantly rich in AGEs exhibit a down regulation of AGE-R1 receptor [109] and have a concurrent increase in plasma isoprostanol, and renal RAGE expression. These effects are ameliorated when a diet low in AGE content is administered [110].

*In-vitro*, the over-expression of AGE-R1 in murine mesangial cells drives increases in AGE binding and in parallel, decreases RAGE and RAGE mediated signalling *via* NF- $\kappa$ B [111]. Moreover, knock down of AGE-R1 with siRNA, reinstated MAPK activity, a key molecule in the inflammatory processes associated with diabetic nephropathy [111]. The protective effects of AGE-R1 in murine mesangial cells, has been hypothesized to be driven *via* modulation of endothelial growth factor receptor (EGFR). EGFR, belongs to a large family of tyrosine kinase receptors, involved in numerous cellular activities, including, growth, differentiation and survival [112].

Clinically, AGE-R1 expression in lymphoblasts from type 1 diabetes patients with severe diabetic nephropathy has also been shown to be reduced relative to age-matched controls [53].

The contribution of AGER3 to the development and progression of diabetic nephropathy has not been extensively researched. However, AGE-induced increases in the expression of AGE-R3 has been demonstrated in cultured endothelial cells and within renal tissues in the diabetic milieu [57, 113]. Paradoxically, AGE-R3 deficient mice develop more severe renal disease and have marked increases in renal AGE deposition [57]. Furthermore, this was associated with albuminuria, mesangial expansion and fibrosis within the kidney cortex. Importantly, the deletion of AGE-R3 was also associated with a decrease in AGE-R1 and increased expression of RAGE demonstrating the existence of AGE-receptor cross talk. This study highlights that the role of AGE-R3 in the

clearance of AGEs [114] is likely more important in diabetic nephropathy than its ability to modulate immune function.

### RECEPTOR-MEDIATED EXPRESSION AND ACTIVATION OF CYTOKINES

The ligation of AGEs to RAGE and its downstream effects are the best characterised of the AGE/AGE-receptor interactions. Binding of AGE to RAGE, stimulates the increased transcription and expression of a number of cytokines and growth factors including monocyte chemoattractant protein (MCP-1), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 (IL-1), platelet derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), interferon- $\gamma$  (IFN- $\gamma$ ), and adhesion molecules (intracellular adhesion molecule (ICAM) [41]. Increased renal RAGE expression associated with diabetes, drives a concurrent increase the expression of MCP-1 [111]. Moreover, urinary MCP-1 expression correlates with albuminuria and is thought to contribute to the initiation and progression of diabetic nephropathy in diabetic patients by recruitment of inflammatory cells [115]. Telmisartan, an angiotensin II receptor blocker, induces a decrease in RAGE expression, superoxide production and MCP-1 expression in mesangial cells *via* the modulation of peroxisome proliferators-activated receptor- $\gamma$  (PPAR- $\gamma$ ). Engagement of AGEs to RAGE, in podocytes, transduces the activation of extracellular signal-regulated kinase (ERK) and NF- $\kappa$ B dependent pathways [116]. Subsequently this drives the production of inflammatory markers, such as MCP-1 and the generation of reactive oxygen species (ROS) [103]. Moreover, inhibition of the ERK pathway (PD98059) and NF $\kappa$ B pathway (mithramycin and parthenolide) can ameliorate these effects [103]. In proximal tubule cells, binding of AGEs and subsequent NF- $\kappa$ B activation increases the pro-inflammatory cytokine, IL-6 [38]. It is well documented that treatment of diabetic rats with ACE-inhibitors, decreases IL-6, IL-1 and TNF- $\alpha$  along with a concurrent reduction in kidney weight and urinary albumin excretion demonstrating that inflammation is an important component of diabetic nephropathy [117]. HMG-CoA inhibitors, collectively called statins, are considered to possess anti-inflammatory and endothelial protective actions in addition to their cholesterol lowering and recently documented renoprotective effects [118].

In addition to the increased expression of numerous cytokines, the engagement of AGEs to RAGE increases intracellular ROS generation [41, 119, 120]. Excess generation of ROS is also an important contributor to the pathogenesis of diabetic nephropathy [121]. In contrast, AGE-R1 is thought to act as a counterbalance to the RAGE induced production of ROS. Indeed, suppression of AGE-R1 further exacerbates the production of intracellular ROS by phosphorylating Ser-36 of p66<sup>shc</sup> leading to suppression of manganese superoxide dismutase (Mn-SOD) activity [122].

### RECEPTOR MEDIATED ACTIVATION OF SIGNALING PATHWAYS

In addition to the increased expression of a number of cytokines, AGE-receptor interactions also stimulate cellular signalling [123, 124]. The signalling pathways which are transduced as the result of binding of AGEs to RAGE include: ERK1/2(p44/p42) MAP kinases, JNK MAP kinases,

PI3 kinase and JAK/STAT pathways [27, 41]. Activation of signalling transduction pathways such as MAPK and JAK/STAT is thought to be fundamental in the development and progression of diabetic nephropathy. Engagement of AGE to RAGE transduces the activation of ERK1/2 MAPK pathways which drives the tubular epithelial-myofibroblast transdifferentiation in diabetic nephropathy [125], postulated to exacerbate fibrosis. Type 2 diabetic patients with diabetic nephropathy have been previously shown to have increased activity of extracellular signal-regulated (ERK) and MAP-kinases compared to healthy controls [126].

The JAK/STAT pathway is known to signal for a number of cytokines and growth factors in many cellular processes including apoptosis, proliferation and differentiation [118]. Treatment of diabetic rats, with statin therapy (Fluvastatin), ameliorated the increased activity of JAK/STAT pathway in the context of renoprotection [118].

AGE-R1 is also a potent inducer of a number of signalling pathways. *In vitro*, AGE-R1 has been shown to activate MAPK, EGF-R and Ras in mesangial cells [111, 112]. These signalling pathways are all involved in cell survival, which is essential for protection against the development and progression of diabetic nephropathy.

The common down-stream effector molecule for these signalling pathways transduced by the binding of AGEs to either RAGE or AGE-R1, is NF $\kappa$ B [16, 41]. NF $\kappa$ B is a pro-inflammatory molecule, which resides in the cytoplasm of resting cells. Upon activation, it translocates into the nucleus. Nuclear translocation of NF $\kappa$ B leads to the transcription of numerous target genes including cytokines, adhesion molecules and anti-apoptotic genes [41, 127]. The constitutive activation of the NF $\kappa$ B and its downstream targets are considered to be central to the inflammatory stress response characteristic of progressive diabetic nephropathy [128].

### MODULATION OF AGE-RECEPTORS FOR THE TREATMENT OF DIABETIC NEPHROPATHY

The interaction between AGEs and their numerous receptors leads to both positive and negative outcomes. There is no doubt that the balance between the expression of these receptors and their down-stream signalling pathways, determines the ultimate outcome. Binding of AGEs with the "clearance" receptors promotes their degradation and removal *via* the kidney thus decreasing their deleterious effects. In contrast engagement of AGEs with their pro-inflammatory receptors such as RAGE, drives the activation of a number of proinflammatory signalling pathways, which are thought to promote the development and progression of diabetic nephropathy.

The modulation of RAGE, both cell surface and soluble has been extensively investigated. Although RAGE is an appealing and relevant contributor to the pathogenesis of diabetic nephropathy, it may not be a valid therapeutic target, since its role in innate immunity may be fundamental to our normal physiological function. However, sRAGE, thought to be the decoy receptor for RAGE ligands such as AGEs, thus inhibiting their ligation with cell surface bound RAGE, has proven beneficial in experimental models of diabetic nephropathy [129]. In type 1 diabetes, circulating levels of both

sRAGE and esRAGE correlate with circulating CML levels [130, 131]. Furthermore, decreases in circulating sRAGE levels predict the degree of renal dysfunction in type 1 diabetic patients [41, 131]. Our own group has previously shown that treatment with perindopril, an angiotensin converting enzyme (ACE)-inhibitor not only improves renal function in type 1 diabetic patients with nephropathy, but also increases circulating sRAGE levels [131]. It is evident that strategies to increase circulating levels of sRAGE may be worth investigating in type 1 diabetic patients with renal disease. Indeed clear renoprotection is also afforded in experimental models of diabetic nephropathy with administration of sRAGE [101]. However, the applicability of sRAGE as a therapy for diabetic nephropathy in type 2 diabetes remains to be determined, since in this group of patients, circulating levels of sRAGE are elevated with worsening renal function [49, 132]. In addition, since sRAGE is a protein, the immunogenicity of this molecule if exogenously administered is unknown.

Recently, a number of other novel treatments which inhibit RAGE have been identified including low-molecular weight heparin and neutralising anti-RAGE antibodies [97, 106, 133-135]. The administration of low molecular weight heparin to block RAGE signalling, warrants further investigation, although patient profiles would have to be carefully researched for possible contraindications. Treatment with anti-RAGE antibodies, normalises creatinine clearance and urinary albumin excretion in experimental diabetic nephropathy [136]. In addition, neutralising RAGE antibodies, prevent RAGE induced elevations in TGF $\beta$ 1 which reduces fibrosis in the kidney, characteristic of diabetic nephropathy [135, 137]. Therefore a humanised anti-RAGE antibody may provide some therapeutic protection for patients with advanced DN.

Our group has previously reported that treatment with cross-link breaker ALT-711, reduces RAGE expression in renal tissue and is renoprotective [93]. We have also identified that ALT-711 has effects on RAGE signal transduction and anti-inflammatory effects [138] in macrovascular disease but this has not yet been confirmed in diabetic kidney disease. This agent is undergoing stage IIb clinical trials in type 1 diabetic patients with renal impairment ([www.alteon.com](http://www.alteon.com)).

*In vitro*, the anti-hyperglycaemic agent, metformin, can down-regulate RAGE expression and signalling in endothelial cells induced by ROS [97]. In type 2 diabetic patients, other anti-glycaemic agents, the thiazolidinediones and the anti-lipidaemic compound, simvastatin can also modulate RAGE expression in macrovascular diabetic complications [137, 139, 140]. These agents have already shown promise in preventing progression in clinical diabetic nephropathy [141, 142].

Dietary interventions, to reduce AGE intake and improve receptor profiles, have also been found beneficial in patients with DN [143-145] in particular with regards to reinstating AGE-R1 expression to control levels. The reduced renal and white blood cell expression of AGE-R1 as seen in diabetes, suggest that AGE-R1 could be a viable target for therapy. Indeed, mice which have a transgenic over expression of AGE-R1 do not develop DN [146]. Since increasing the levels of AGE-R1 in renal cells produces a concomitant down

regulation of RAGE, targeting of AGE-R1 would likely provide dual benefits. This may also be the case for AGE-R3, which has roles in apoptosis, activation, inflammation and tumour growth. With its increased expression in diabetic nephropathy, and it is highly likely, that as AGE-R1 is down-regulated, AGE-R3 expression increases to clear the increased AGE load. Whilst we would not want to delete AGE-R3 completely, as this enhances the progression of diabetic nephropathy, it is likely, that if we promote the increased expression of AGE-R1 there will be a concurrent decrease in AGE-R3 as a counter-balance. Furthermore, genetic deletion of AGE-R3 in mouse models worsens diabetic nephropathy and interestingly decreases the expression of AGE-R1 while increasing the expression of RAGE [57]. The value of AGE-R3 as a direct target for intervention remains to be determined.

## CONCLUSION

In pathological conditions, such as diabetes, characteristic metabolic and hemodynamic factors provide an environment which pathologically alters the levels of AGE-receptors i.e. increased levels of RAGE and AGE-R3 in the context of a down-regulation of AGE-R1 and sRAGE. Indeed, it is likely, that a fine balance exists between the levels of these pro and anti-inflammatory receptors, which determines whether diabetic complications, such as nephropathy develop and subsequently progress.

In conclusion, modulation of AGE-receptors, specifically AGE-R1 and sRAGE hold potential as valid targets for the treatment of diabetic nephropathy and warrant further investigation.

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