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Receptor for advanced glycation end-products (RAGE) and soluble RAGE (sRAGE): cardiovascular implications

JASON B LINDSEY, FRANCESCO CIPOLLONE, SHUAIB M ABDULLAH, DARREN K MCGUIRE

Abstract

Disorders of glucose metabolism are associated with increased risk for cardiovascular disease (CVD) complications, including coronary, peripheral and cerebral arterial disease, that account for the majority of morbidity and mortality among patients with diabetes mellitus (DM). These associations between glucose and CVD risk extend continuously well below the glycaemic thresholds established for the diagnosis of diabetes, including significantly increased risk associated with impaired fasting glucose, impaired glucose tolerance, and even high normal glucose concentrations. While these epidemiological observations have established a clear association between cardiovascular disease and dysglycaemia and suggest a direct causal link, the mechanisms by which hyperglycaemia may contribute to the development, progression and instability of atherosclerosis remain unclear. A number of recent advances in the realm of vascular biology have identified several novel, plausible pathways that might link hyperglycaemia with atherosclerosis, individually or in aggregate. Key among them are the interaction between advanced glycation end-products (AGEs) and the receptor for AGEs (RAGE), which exists as a trans-membrane signalling receptor and as a circulating form, soluble RAGE (sRAGE).

The purpose of this review is to provide an overview of the present understanding of RAGE and sRAGE, their plausible role linking perturbed glucose metabolism with the development, progression and instability of atherosclerosis, and the potential thera-

peutic implications of modulation of this biological system.

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Key words: advanced glycation end products, atherosclerosis, diabetes, hyperglycaemia, receptor for advanced glycation end products.

Introduction

The prevalence of diabetes mellitus (DM) is increasing worldwide and macrovascular disease remains the leading cause of morbidity and mortality among diabetic patients.¹ Randomised trials have demonstrated the benefit of intensive glycaemic control on microvascular disease risk associated with DM,^{2–4} but the effect of glycaemic control on macrovascular disease risk remains less well defined.^{2,5–9} The University Group Diabetes Program (UGDP),⁶ the Veterans' Administration Cooperative Study of Diabetes,¹⁰ the United Kingdom Prospective Diabetes Study (UKPDS 33)² and a meta-analysis of studies of rosiglitazone⁹ all failed to demonstrate statistical benefit associated with intensification of glycaemic control with regard to diabetic macrovascular clinical events. Conversely, the metformin randomised sub-study of the UKPDS⁷ and the PROspective pioglitAzone Clinical Trial In macroVascular Events (PROACTIVE) study⁸ demonstrated significant improvements in selected cardiovascular disease (CVD) risks with glucose-modulating therapies. Results from the Epidemiology of Diabetes Interventions and Complications (EDIC) long-term observational extension of the Diabetes Control and Complications Trial (DCCT) demonstrated a statistically significant reduction in CVD events among patients who received intensive treatment during the randomised controlled portion of the trial compared with 'conventional' therapy.^{5,11} However, the validity of the EDIC study findings is uncertain given the limited power derived from the inclusion of 'soft' end points such as need for revascularisation and incident angina in the principal composite analyses; the low overall number (n=36) of sentinel 'hard clinical events (CVD death, myocardial infarction and stroke); and the observational nature of the extended follow-up period off-protocol. Most recently, two large randomised clinical outcomes trials specifically designed to assess the effect of intensive glucose control in cohorts with type 2 diabetes at increased CVD risk failed to demonstrate improvements in any CVD outcome.^{12,13}

Given the lack of success at materially affecting CVD risk

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in the studies of glycaemic control reported to date, investigations are accelerating to identify molecular targets of intervention to complement glucose control, focusing on the pathobiological interplay between hyperglycaemia and atherosclerosis. Myriad candidate mechanisms are being explored in an attempt to understand better the excess vascular disease observed in diabetic patients. Included among these plausible mechanistic processes are the roles of adiponectin, C-peptide, the thromboxane receptor, plasminogen activator inhibitor-1, and many others.^{11,14} However, this review will focus on one of the more promising and rapidly advancing areas of research, the interaction and downstream effects of the ligand-RAGE axis.

Advanced glycation end-products

Non-enzymatic glycation of proteins and lipids occurs with ageing, a process that is markedly accelerated in the setting of glucose dysregulation, such as diabetes mellitus and oxidative stress.^{15,16} Protein glycation occurs via a non-enzymatic reaction between the amino group of the protein and carbonyl group of a reducing sugar to form an unstable Schiff base, which is rapidly converted to a more stable Amadori product.¹⁷ These glycated proteins in aggregate are referred to as advanced glycation end-products (AGEs), and the resultant alteration of protein structure adversely affects their function and degradation.

AGEs have been implicated in a number of pathological processes associated with micro- and macrovascular disease complications in diabetes.¹⁵ AGEs promote protein cross-linking, which alters protein structure and function, leading to the covalent trapping of pro-atherogenic particles such as low-density lipoprotein (LDL) in the arterial wall.¹⁸ Additionally, formation of AGEs and their interaction with proteins (type I collagen and elastin) in the extracellular matrix (ECM) of the vascular wall lead to an increase in ECM area, with a resultant decrease in vascular compliance.^{19,20} Another mechanism by which AGEs may contribute to atherogenesis is via the generation of reactive oxygen species (ROS).²¹ The contribution of oxidative stress and ROS to the development of atherosclerosis is well established.²² AGEs have been demonstrated to enhance neutrophil respiratory burst activity via NADPH oxidase, with subsequent augmentation in ROS production;²³ this AGE-mediated increase in oxidative stress may contribute to accelerated atherosclerosis in animal models of atherosclerosis.²⁴ Numerous AGEs have been identified, including glycosylated haemoglobin, that reflects intermediate-term glycaemic exposure and is the gold-standard parameter for optimising the treatment of diabetes. The predominant circulating AGEs are carboxymethyl-lysine (CML) adducts of proteins.²⁵⁻²⁷ CML adducts are formed from both glycoxidation of proteins and lipid peroxidation;²⁸ CML accumulation has been demonstrated in vascular tissue and in atherosclerotic lesions from animal models of diabetes and humans.²⁹⁻³¹ In addition to CML, AGEs are present in coronary atheroma in both diabetic and non-diabetic animals;³² concentrations of circulating AGEs correlate with the severity of coronary artery disease and with adverse clinical outcomes;³³ and AGE concentrations are also elevated in non-diabetic patients with coronary disease.³⁴

In addition to the extracellular interactions of AGEs, selected AGEs act as specific ligands for a membrane-bound receptor, the receptor for advanced glycation end-products (RAGE), that was first isolated and characterised in 1992.³⁵ Interaction between CML, the most prevalent circulating AGE *in vivo*, and RAGE results in enhanced transcription and production of various pro-inflammatory mediators via activation of nuclear factor (NF)- κ B in endothelial cells, vascular smooth muscle cells and mononuclear phagocytes both *in vitro* and *in vivo*.^{36,37} These RAGE-dependent pro-inflammatory genes include: intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, tumour necrosis factor- α (TNF- α), interleukin-1 (IL-1), interleukin-6 (IL-6) and cyclo-oxygenase-2 (COX-2).³⁶⁻³⁸ In aggregate, these observations support the important pathobiological effects of AGE-RAGE interaction through modulation of a number of processes related to inflammation and oxidative stress, all of which have been implicated in the formation, progression and instability of atherosclerosis with resultant macrovascular complications.

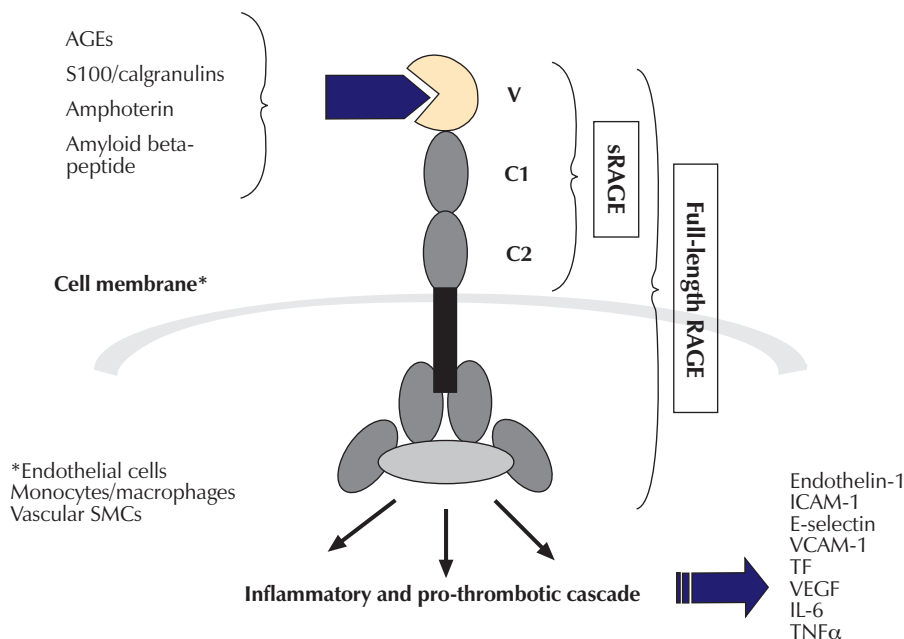
Other RAGE ligands

Other important RAGE ligands, the S100/calgranulin polypeptides and high mobility group box-1 (HMGB1),³⁹ have been identified that bind to and subsequently activate the receptor. These ligands may prove more important than AGE-RAGE binding to the development, progression and instability of the atherosclerotic process in patients both with and without diabetes. In animal models of diabetes, S100/calgranulins and HMGB1 have both been demonstrated to accumulate within the vasculature in settings of glucose dysregulation and oxidative stress, and have pro-inflammatory characteristics.³⁹⁻⁴¹ Another ligand of RAGE, the RAGE binding protein (EN-RAGE) or S100A12,³⁹ is a member of the S100-calgranulin family of cytokines and has been implicated via interactions with RAGE to activate a pro-inflammatory cascade via IL-1 β and TNF α . Additionally, in animal models interaction between S100A12 and RAGE resulted in the upregulation of transcription of NF- κ B and increased expression of VCAM-1 and ICAM-1.³⁹ Notably, these downstream effects of S100A12/RAGE binding were ameliorated by blocking RAGE with anti-RAGE immunoglobulin G (IgG) or by treatment with sRAGE, thus implicating RAGE modulation as a potential new strategy for the prevention of atherosclerosis.

Receptor for advanced glycation end-products

RAGE is a multi-ligand member of the immunoglobulin superfamily of cell surface molecules that is expressed in a variety of cell lines, including endothelial, neuronal, smooth muscle, mesangial and mononuclear (figure 1).^{42,43} Given the consistent observations of the association between ligand-RAGE interaction and modulation of a large variety of mediators known to influence atherosclerotic disease development, progression and instability, a number of investigations have extended these observations by assessing the influence of RAGE on atherosclerosis parameters. Furthermore, RAGE may play a role in other diverse disease processes such as Alzheimer's disease, rheumatoid arthritis, chronic kidney

Figure 1. Summary of the AGE-RAGE-sRAGE axis. Ligand binding to RAGE results in enhanced transcription of several pro-inflammatory and pro-thrombotic molecules, which are also known to promote atherosclerosis. sRAGE contains the ligand-binding region of RAGE (V-domain) and may serve as a molecular decoy



Key: ICAM-1 = intercellular adhesion molecule-1; VCAM-1 = vascular cell adhesion molecule-1; TF = tissue factor; VEGF = vascular endothelial growth factor; IL-6 = interleukin-6; TNF- α = tumour necrosis factor-alpha; SMC = smooth muscle cell; AGEs = advanced glycation end-products; RAGE = receptor for advanced glycation end-products; sRAGE = soluble RAGE

disease and inflammatory bowel disease;⁴⁴ however, this discussion is beyond the scope of this review.

In animals, a number of studies have demonstrated a link between RAGE and atherosclerosis. Bucciarelli *et al.* investigated the role of RAGE in diabetic apolipoprotein (apo) E-null mice,⁴⁰ which are prone to develop atherosclerosis. In this model, they demonstrated that ligand-RAGE binding resulted in accelerated atherosclerotic lesion progression in both diabetic and non-diabetic apoE-null mice. In a similar model, Kislinger *et al.* provided evidence that ligand-RAGE co-localisation was most pronounced in aortic tissue from diabetic mice with advanced atherosclerosis.⁴⁵ Subsequent interruption of ligand-RAGE binding resulted in marked diminution of RAGE expression and generation of pro-inflammatory adhesion molecules (VCAM-1 and tissue factor). These findings support the hypothesis that macrovascular atherosclerosis in diabetic mice is, in part, mediated by activation of RAGE.

Recently, additional supportive data have been published implicating RAGE in vascular perturbation.⁴⁶ Compared with apolipoprotein E knock-out (apoE^{-/-}) mice with intact expression of RAGE, mice with double knockout of apoE and RAGE (apoE^{-/-}/RAGE^{-/-}) developed significantly less atherosclerosis. Further, the double-knockout mice displayed evidence of improved endothelial function, with enhanced endothelium-dependent response to acetylcholine administration, when compared with apoE^{-/-} mice. These observations may be explained in part by the demonstration that in the absence of RAGE expression (apoE^{-/-}/RAGE^{-/-}), mice have significantly lower activity of numerous

inflammatory mediators, namely endothelial-bound and soluble VCAM-1, monocyte chemoattractant peptide-1 (MCP-1), interleukin-10, CD40 and S100/calgranulins. These important observations provide further evidence that in an environment conducive to atherogenesis and progression such as in the apoE^{-/-} mouse model, the ligand-RAGE axis appears to play an important role in vascular pathology and development of atherosclerotic burden, even in the absence of diabetes.

Similar observations are derived from studies executed in a mouse model with streptozotocin-induced diabetes.⁴⁷ Diabetic apoE^{-/-}/RAGE^{-/-} mice had significantly less atherosclerotic plaque accumulation than diabetic apoE^{-/-} mice with intact RAGE expression and had lower levels of pro-inflammatory mediators including NF- κ B subunit p65, VCAM-1 and MCP-1. These two studies in aggregate provide incremental evidence that RAGE is involved in perturbation of normal vascular function and that it is also involved in the development of atherosclerosis in both non-diabetic and diabetic environments.

In addition to atherosclerosis development and progression, ligand-RAGE interaction has been implicated in modulation of restenosis following percutaneous arterial interventions. Compared with non-diabetic patients, patients with DM are at increased risk for restenosis and resultant need for repeat revascularisation following percutaneous coronary interventions (PCI),^{48,49} due to exaggerated neointimal proliferation at the site of vascular injury in the setting of diabetes, which has been demonstrated in both animal models and clinical studies.^{48,50,51} Using animal models of balloon-

mediated arterial injury, which correlate closely with clinical restenosis, the ligand-RAGE axis appears to modulate the neointimal response.^{52,53} In a rat model of carotid arterial injury, compared with non-diabetic rats, diabetic rats had increased accumulation of AGE, S100/calgranulin and RAGE within the neointima and media in response to balloon injury; and blockade of RAGE/ligand binding by administration of sRAGE led to decreased vascular smooth muscle cell proliferation and suppressed neointimal formation with a resultant increase in luminal area in both diabetic and non-diabetic rats.⁵² Similarly, in a mouse model using arterial denudation as the vascular injury, the neointimal response was significantly diminished in RAGE-null mice and in wild-type mice administered sRAGE, providing strong support for the key contribution of RAGE to the vascular response to acute vessel injury.⁵³ Taken together, these studies suggest that RAGE activation plays a pivotal role in neointimal formation and that in addition to atherosclerosis reduction, ligand-RAGE modulation may have other therapeutic implications such as reduction of neointimal hyperplasia and restenosis after angioplasty.

More recently, multiple studies have provided support for a central role of ligand/RAGE interactions in myocardial ischaemia/reperfusion injury.⁵⁴⁻⁵⁷ While the validity of these observations and their clinical relevance remain to be defined, if true they would suggest that myocardial ischaemia and infarction may represent yet another potential target for therapeutic modulation.

RAGE may also be involved in mediating the beneficial effects of the diabetic medication, rosiglitazone, a peroxisome proliferator-activated receptor γ (PPAR γ) agonist belonging to the thiazolidinedione (TZD) class.⁵⁸ In fact, administration of rosiglitazone to rat smooth muscle cells decreased RAGE expression and decreased cell proliferation *in vitro*. Furthermore, in a carotid arterial injury model, diabetic rats receiving rosiglitazone had significantly less neointimal formation and RAGE expression compared with non-diabetic rats.⁵⁸ All these findings suggest that PPAR γ activation may reduce neointimal hyperplasia after vascular injury through a RAGE-dependent mechanism.

How TZDs may modulate RAGE expression has not yet been elucidated. However, Marx *et al.* investigated the effects of the two TZDs presently available, rosiglitazone and pioglitazone, on RAGE expression in cultured human umbilical vein endothelial cells (HUVECs).⁵⁹ Exposure of HUVECs to either TZD resulted in a similar reduction in RAGE cell surface expression. Furthermore, TZDs reduced RAGE mRNA expression via inhibition of NF- κ B activation. Thus, this study demonstrated, at least in part, how TZDs may influence RAGE expression and its deleterious inflammatory activity in subjects with DM. However, further investigation is warranted into the complex interaction of TZDs and RAGE in humans.

Several lines of evidence support an important role of RAGE in human atherosclerosis and plaque instability. Analysis of carotid plaques obtained from diabetic and non-diabetic patients undergoing endarterectomy,³⁸ as well as analysis of coronary plaques from patients who died suddenly, revealed that atherosclerotic plaques from patients

with DM had significantly more RAGE expression compared with specimens from non-diabetic subjects. In addition, the plaque expression of RAGE co-localised with S100A12, activated NF- κ B, cyclo-oxygenase-2/inducible prostaglandin E synthase-1 (COX-2/mPGES-1), matrix metalloproteinase-2 and -9 (MMP-2 and MMP-9) expression, smooth muscle cell apoptosis and macrophage infiltration, all factors that have been associated with plaque instability. This suggests that the over-expression of RAGE in diabetic plaques may promote plaque instability and rupture.

RAGE expression in inflammatory cells infiltrating atherosclerotic plaques is a function of glycaemic control in diabetic patients.³⁸ Nevertheless, evidence for modulation of RAGE expression in atherosclerotic plaques by a glucose-independent mechanism has been reported recently.⁶⁰ Cucurullo and colleagues randomised 70 type 2 diabetic patients with asymptomatic carotid artery stenosis (> 70%) to an American Heart Association (AHA) step 1 diet plus simvastatin (40 mg/day) or to AHA step 1 diet alone for four months before carotid endarterectomy. After endarterectomy, carotid plaque specimens were evaluated for expression of AGEs, RAGE, NF- κ B, MMP-2 and MMP-9. Expression of RAGE was significantly reduced in carotid plaques from those diabetic patients randomised to simvastatin compared with those randomised to diet alone. Additionally, MMP activity was significantly decreased, with a concomitant increase in plaque collagen content, suggesting the presence of a more stable plaque in the simvastatin-treated subjects. These important, hypothesis-generating findings suggest an important role of RAGE in plaque stabilisation, and for the first time demonstrate that RAGE blockade may be an effective target for plaque stabilisation in humans.

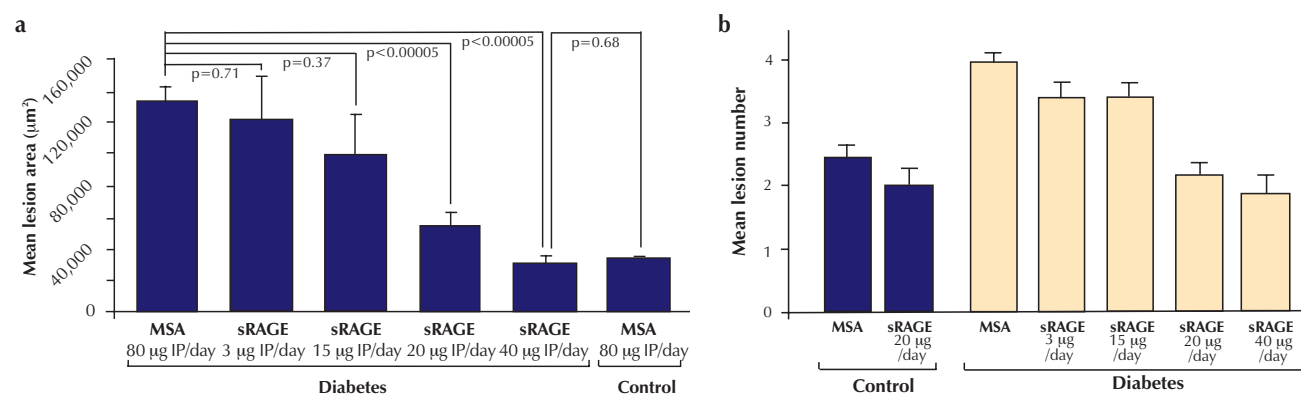
In short, multiple lines of evidence suggest that downstream actions of RAGE activation play a role in the pathogenesis of atherosclerosis and atherothrombosis (complicated atherosclerosis) in diabetes. These downstream actions include: increased oxidative stress, a marked pro-inflammatory state, upregulation of adhesion molecules and neointimal proliferation; all of which lead to the generation and progression of atherosclerosis. Future attempts to disrupt the RAGE/ligand axis by pharmacological means may provide another modality to treat the epidemic of vascular disease that we are now confronting.

The soluble receptor for advanced glycation end-products

The soluble form of RAGE that exists in the circulation, sRAGE, is a product of both alternative splicing of the gene for RAGE (esRAGE) and cleavage of membrane-bound RAGE.^{61,62} While the biological function of sRAGE has not been clearly defined, one proposed pathological role is as a competitive inhibitor of ligand-RAGE interaction and the subsequent downstream signalling, as described previously. Furthermore, sRAGE may also serve as a scavenger receptor for circulating AGEs and other RAGE ligands.⁶³ The biological importance of sRAGE within the ligand-RAGE axis is only recently beginning to be understood.

A series of studies has been published assessing the role of sRAGE in different animal models of atherosclerosis. Park

Figure 2. sRAGE suppresses accelerated diabetic atherosclerosis. (a) Mean atherosclerotic lesion areas (μm^2) were determined in diabetic or control mice treated as indicated (horizontal axis). The results of statistical analyses are shown. There were no statistically significant differences between diabetic mice and diabetic mice treated with MSA. Diabetic mice treated with MSA, $n=10$; sRAGE at $3\ \mu\text{g}/\text{day}$, $n=5$; sRAGE at $15\ \mu\text{g}/\text{day}$, $n=5$; sRAGE at $20\ \mu\text{g}/\text{day}$, $n=8$; sRAGE at $40\ \mu\text{g}/\text{day}$, $n=6$; controls treated with MSA, $n=11$. (b) Total lesion number per mouse was determined from analysis of sections two to five prepared from the aortic sinus. Treatment with MSA, diabetic mice versus control, $p<0.0001$. Diabetic mice treated with MSA versus sRAGE at $3\ \mu\text{g}/\text{day}$, $p=0.09$; at $15\ \mu\text{g}/\text{day}$, $p=0.09$; at $20\ \mu\text{g}/\text{day}$, $p<0.0001$; and at $40\ \mu\text{g}/\text{day}$, $p<0.0001$. Control treated with MSA versus diabetic mice treated with sRAGE at $20\ \mu\text{g}/\text{day}$, $p=0.30$; and at $40\ \mu\text{g}/\text{day}$, $p=0.12$



Key: sRAGE = soluble receptor for advanced glycation end-products; MSA = mouse serum albumin

Reproduced with permission from: Park L *et al.* *Nature Medicine* 1998;4:1025-31

et al. studied the effect of exogenous administration of sRAGE in apoE-deficient mice, a mouse model of atherosclerosis, and in that investigation, also rendered the mice diabetic with streptozotocin.⁶⁴ Treatment of these mice with a daily infusion of sRAGE initiated at the time of streptozotocin administration retarded the development and progression of atherosclerosis in a dose-dependent fashion, independent of plasma levels of glucose and lipids (figure 2). Remarkably, at the highest dose of sRAGE infused, there was no detectable increment in atherosclerosis burden in the diabetic mice compared with the non-diabetic control mice. The findings of Park and colleagues are important because, by the administration of sRAGE and the successive diminished atherosclerotic burden, they provide critical evidence for the potential importance of the ligand-RAGE axis in diabetic vascular disease, and identify it as a potential target for future pharmacological strategies aimed at atherosclerosis prevention.

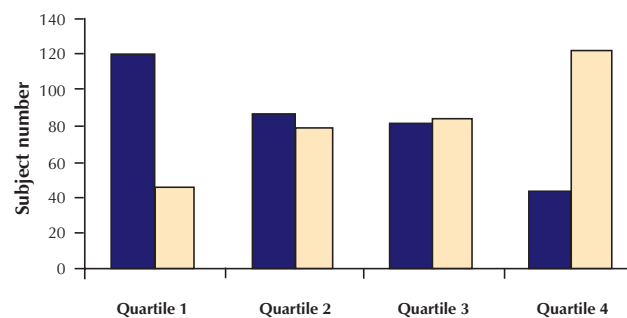
Using the model developed by Park and colleagues, Bucciarelli *et al.* further investigated the effects of sRAGE administration on established atherosclerosis in diabetic, apoE-null mice.⁴⁰ Likewise, they demonstrated that atherosclerosis progression, as quantified by mean atherosclerotic lesion area and number of complex lesions, was halted by administering sRAGE. Further, to determine plausible mechanistic effects of sRAGE administration, Bucciarelli *et al.* measured parameters of inflammation and the expression of pro-thrombotic mediators. Compared with diabetic mice receiving albumin (placebo), those receiving sRAGE had significantly diminished activity of MCP-1, COX-2, VCAM-1 and MMP-9 within aortic tissue. The decreased expression of these pro-atherogenic mediators all correlated with significantly lower plaque expression of RAGE in sRAGE-treated

mice. These data provide further provocative evidence of a crucial role for sRAGE, not only in the development of atherosclerosis but also its progression in the diabetic vasculature.

Extending these findings, Wendt and colleagues developed a different model of diabetic atherosclerosis associated with hyperglycaemia in the setting of insulin resistance.⁶⁵ They bred apoE-deficient mice that were also db/db-deficient, a genotype that leads to an insulin resistance animal model of type 2 diabetes and is associated with accelerated atherosclerosis. Compared with non-diabetic apoE-null mice, diabetic apoE-null mice had significantly more atherosclerosis; those diabetic mice receiving sRAGE had marked attenuation of the degree of atherosclerosis to an amount commensurate with non-diabetic mice. The mice receiving sRAGE not only had less atherosclerosis; they also had significantly lower levels of VCAM-1 and tissue factor in aortic tissue, and antigen/activity of MMP-9 was also significantly lower in those treated with sRAGE. These data further strengthen the inverse correlation between sRAGE and atherosclerosis. These findings together elegantly demonstrate the potential influence of the interruption of the ligand-RAGE axis on the development and progression of atherosclerosis; they support a potential therapeutic role for sRAGE and other modulators of ligand-RAGE interaction, and warrant further investigation.

To date, few studies have been performed to evaluate the function of sRAGE in human atherosclerosis. A recent study by Falcone *et al.* was the first to attempt to better define the role of sRAGE in humans.⁶⁶ In their case-control study of middle-aged, Italian, non-diabetic men, plasma sRAGE levels were lower in men with coronary artery disease than in those without coronary artery disease (figure

Figure 3. Lower prevalence of coronary artery disease with increasing plasma sRAGE levels. The number of subjects is shown across increasing quartiles of sRAGE levels. Coronary artery disease cases (purple bars) and controls (yellow bars). Coronary artery disease cases are defined as the presence of ≥ 1 coronary artery stenosis $> 75\%$ at catheterisation. Controls represent age-matched controls with no history of angina or heart disease, a normal resting electrocardiogram, and normal exercise ECG stress testing



Key: sRAGE = soluble receptor for advanced glycation end-products

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Arterioscler Thromb Vasc Biol 2005;**25**:1032-7

3). This was the first study to demonstrate an inverse association between plasma sRAGE levels and coronary artery disease in non-diabetic men. Although their findings are provocative, there are many limitations to their study. Mainly, the population studied was a very discrete, homogeneous population and therefore the generalisation of their findings to other multi-ethnic populations is of uncertain validity. Secondly, the assay used was a non-specific assay for sRAGE that may not have accurately quantified the aggregate concentration of sRAGE comprised of multiple splice variants and the cleaved form of membrane-bound RAGE. Despite these and other limitations, this early work sets the stage for future, larger investigations which will hopefully better define the biological role of sRAGE in human atherosclerosis.

A recent small, observational study by Katakami *et al.* of Japanese patients with type 2 DM revealed an inverse relationship between plasma esRAGE levels, the splice variant form of RAGE and glycosylated haemoglobin levels.⁶⁷ Furthermore, higher levels of esRAGE were associated with higher levels of plasma high-density lipoprotein (HDL) in this cohort of patients. Another study found that esRAGE was strongly associated with many of the individual components of the metabolic syndrome, including body mass index and insulin resistance,⁶⁸ and was found to be independently associated with carotid atherosclerosis. In an attempt to understand further the relationship between sRAGE and atherosclerosis in humans, Katakami *et al.* evaluated the association between serum esRAGE levels and carotid intimal-medial thickness (IMT), a measure of subclinical atherosclerosis, in a small ($n=179$) cohort of subjects with type 2 DM.⁶⁹ They showed an inverse relationship between

esRAGE levels and mean carotid IMT and, when using a multivariable model of conventional risk determinants for atherosclerosis, esRAGE levels were independently associated with carotid IMT. The same group of investigators found that low circulating levels of esRAGE were an independent predictor of cardiovascular mortality in a small cohort of both diabetic and non-diabetic individuals with end-stage renal disease.⁷⁰ Although these studies are provocative, they have only investigated the role of esRAGE and not the proteolytically cleaved form of RAGE, which appears to be present and to contribute to the concentration of the circulating form of RAGE.⁶² Furthermore, they are limited by their small sample sizes which limit the depth of multivariable analyses that can be performed. Nevertheless, further investigation into the role played by sRAGE in subclinical atherosclerosis is warranted.

In one of the few studies assessing therapeutic interventions in humans to augment endogenous production of sRAGE, Forbes *et al.* demonstrated that compared with placebo, the angiotensin-converting enzyme inhibitor (ACE-I) perindopril increased human plasma sRAGE levels and reduced plasma AGE concentrations.⁷¹ These findings suggest an additional mechanistic effect of ACE inhibition in treatment and prevention of vascular disease.

All things considered, sRAGE may one day prove to be a useful biomarker for coronary artery disease as well as a pharmacological agent to treat and prevent atherosclerosis. Obviously, further investigation into sRAGE and atherosclerosis is required.

Future directions

Mounting evidence strongly implicates the importance of RAGE and its ligands in the development of vascular disease, including atherosclerosis and restenosis after PCI. The lack of convincing evidence from multiple large trials that strict glycaemic control can reliably prevent macrovascular complications from DM has led the way for investigation of alternative targets for the treatment and prevention of vascular disease in those with DM. One biologically plausible pathway is the ligand-RAGE axis. The interruption of this pathway in animal models by treatment with sRAGE has resulted in stabilisation and even regression of atherosclerosis. Amplification of the endogenous production of sRAGE or exogenously administered sRAGE may provide an alternative therapy to combat the growing epidemic of diabetic vascular disease. Ongoing investigations at several institutions, including our own, will hopefully better elucidate the role of sRAGE in human atherosclerosis. In the meantime, proven therapies to prevent cardiovascular events in diabetic subjects must be rigorously implemented. They include the use of aspirin, statins, aggressive treatment of concomitant hypertension and, probably most important, exercise and weight loss. In the near future we hope to have a better understanding of the reasons for the abundance of diabetic vascular disease and better therapies to both prevent and treat the severe complications.

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Conflict of interest statement

None declared.

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