

## RAGE and cardiovascular disease

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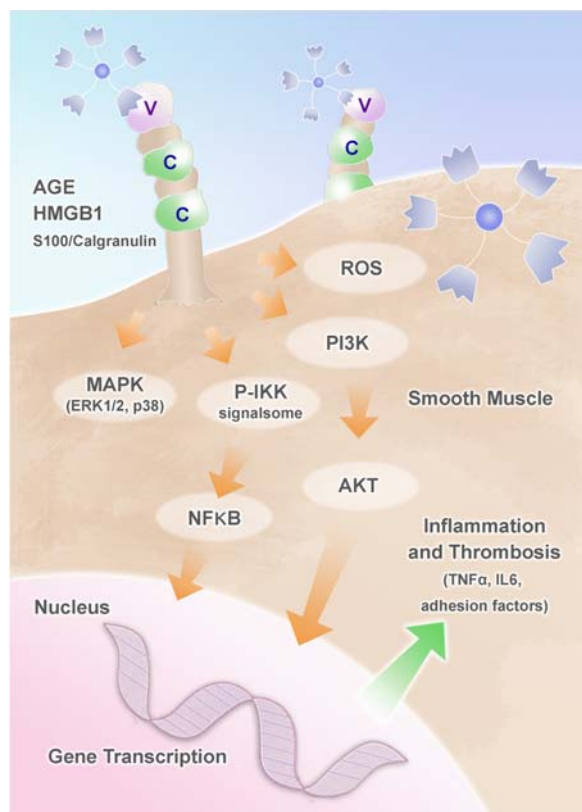
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## 1. ABSTRACT

RAGE is pattern recognizing receptors for diverse endogenous ligands. RAGE activation by RAGE ligands is known to be associated with reactive oxygen species generation, activation of NF kappa B, as well as recruitment of proinflammatory cells. Activated endothelial cells, vascular smooth muscle cells in atherosclerotic plaques and activated inflammatory cells all have increased expression of RAGE, which with its interaction with RAGE ligands increases the secretion of proinflammatory cytokines and cell adhesion molecules. Furthermore, RAGE may have a significant role in leukocyte recruitment into the intima of the atherosclerosis. Initial insults resulting in endothelial dysfunction will result in leukocyte infiltration, oxidative stress and vascular inflammation that is amplified by RAGE activation. RAGE and its interaction with RAGE ligands may be important for initializing and maintaining the pathological processes that result in various entities of cardiovascular disease. Soluble RAGE competitively inhibits the binding of RAGE ligands to RAGE and attenuates the development of atherosclerosis *in vivo*. Thus RAGE may be a promising target for treatment of cardiovascular disease in the future.

## 2. INTRODUCTION

With aging, changes in dietary patterns and increasing sedentary lifestyles, cardiovascular diseases resulting from atherosclerosis have become the most important cause of mortality and morbidity in the general population (1). Although atherosclerosis develops from multiple risk factors such as hypertension, dyslipidemia, diabetes, aging and smoking, the common pathway for its development is endothelial dysfunction and vascular inflammation (2,3). Multiple risk factors including hypertension, diabetes, smoking, and dyslipidemia act in concert to increase oxidative stress and inflammation in vascular walls (1-3). Recent studies have demonstrated that receptor for advanced glycation endproducts (RAGE) and its ligands may play an important role in mediating vascular inflammation and the subsequent development of atherosclerosis (4-6). RAGE is a pattern recognition receptor that interacts with multiple ligands and elicits innate immune responses from leukocytes (1,2). Unlike Toll like receptors (TLRs) that primarily recognize ligands originated from exogenous pathogens, RAGE interacts with endogenous ligands, and has been strongly implicated in the pathogenesis of multiple diseases triggered by chronic



**Figure 1.** Binding of RAGE ligand with RAGE results in activation of various signal cascades such as MAPK activation, increase in reactive oxygen species generation, increased activation of the PI3K-Akt pathway and increased activation of NFκB. The activation of NFκB may increase the expression of TNFα, Interleukin 6 and various cell adhesion molecules, which may act in concert to increase tissue inflammation.

inflammation (2, 3). Endogenous ligands for RAGE, such as high mobility group box 1 (HMGB1), s100/calgranulin, amyloid beta peptide and advanced glycation endproducts (AGEs) are significantly increased during tissue injury and cell necrosis (4-7) and are strongly influenced by environmental factors and aging (8-12). RAGE ligands such as AGE, HMGB1, S100/calgranulin that are involved in the pathogenesis of diabetic complications, amyloidosis, immune/inflammatory disorders and tumor biology interact with RAGE to manifest their biological activity. In this article, we will review the evidence of RAGE in inflammation, role of RAGE in the pathogenesis of cardiovascular disease and the possibility of the therapeutic role of soluble RAGE in cardiovascular disease.

## 3. RAGE SIGNALING IN INFLAMMATION

AGEs are products of non enzymatic glycation and oxidation of proteins and lipids, that result in formation of majors AGEs products such as N-carboxymethyl-lysine (CML), pentosidine and methylglyoxal derivatives (6). The deposition of AGEs on cells and its interaction with RAGE can adversely affect normal cellular physiology. RAGE is a

type 1 membrane protein of the immunoglobulin superfamily and is a pattern recognizing receptors for diverse endogenous ligands. It is expressed abundantly in normal lung tissues, macrophages, T cells, podocytes, Müller cells, glial cells, vascular smooth muscle cells and endothelial cells (13). In pathologic states, the expression of RAGE in the vascular smooth muscle cells and the endothelial cells are highly upregulated (14-16). RAGE activation is known to be associated with reactive oxygen species (ROS) generation, activation of NF kappa B, as well as recruitment of proinflammatory cells (4,17-19) (Figure 1). Also, RAGE activation is involved in activation of myriads of diverse signaling pathways such as ERK 1/2, MAPK, SAPK/JNK, rho GTPase, PI3K and the JAK/STAT pathway (18-26). Studies have shown a direct role for RAGE in inflammatory cell adhesion and recruitment with RAGE being demonstrated to be a counter-receptor for leukocyte integrins, thereby being directly involved in the recruitment of inflammatory cells *in vitro* and *in vivo* (4,27). In a study performed in a mouse thioglycollate-induced acute peritonitis model, recruitment of both neutrophils and macrophage were significantly impaired in RAGE knockout mice compared to the wild type mice, with the recruitment of leukocytes being mediated through the interaction of RAGE with beta 2-integrin Mac-1 (4).

A unique feature of RAGE is its interaction with multiple ligands and the colocalization of ligand-RAGE complexes in sites of ligand accumulation (28,29) (Figure 1). The major ligands for RAGE that have so far been elucidated are AGEs, high mobility group box 1 (HMGB1) and S100/calgranulins (6,30).

AGEs are known to arise both endogenously using endogenous substrates and by exogenous absorption from food (31). Endogenous AGEs are generated by non enzymatic reaction between reducing sugars and amino acid groups residing on proteins, lipids and nucleic acids. Although the reaction is driven largely by hyperglycemia, increase in oxidative stress with resulting oxidation of sugars will result in increased formation of highly reactive mono or dicarbony groups that bind to amino acids and form AGEs (32,33). Activated leukocytes will form AGEs independent of hyperglycemia by producing enzymes such as NADPH oxidase and myeloperoxidase, generating oxidation of amino acids and AGEs (32,34). Increased activation of the aldose-reductase mediated polyol pathways, by generating reactive intermediates such as glyoxal, methylglyoxal or 3-deoxyglucosone, is associated with increased AGE formation (32,35). Endogenously generated AGEs engage with RAGE to upregulate proinflammatory signals via its activation of NF kappa B in endothelial cells, vascular smooth muscle cells and monocytes, which may mediate the pathogenesis of inflammation that are crucial to the development of vascular disease (18,36). Indeed, accumulation of AGEs is increased in diabetes, hyperglycemia, aging, atherosclerotic vascular disease, renal failure and Alzheimer disease (18,37-40).

Exogenous food borne AGEs, such as premelanoidins and melanodins, are derived by Maillard

reaction during heating and processing of food with 5-30% of AGEs consumed being absorbed into the circulation (31,41). In contrast to endogenous AGEs, exogenous AGEs have been suggested to have an antioxidant and antifibrotic effect *in vitro* (31,42). In a study by Ruhs *et al*, the expression of mice cardiac fibroblasts treated with bread crust extracts was associated with significant reduction in the expression of smooth muscle cell  $\alpha$ -actin and tropomyosin-I, suggesting an antifibrotic effect of exogenous AGE *in vitro* (31). However, in a study by Vlassara *et al*, diabetic subjects administered with high AGE diet were associated with increased expression of mononuclear cell TNF-alpha/beta, serum vascular cell adhesion molecule and plasma C reactive protein (43). Also, in a study by Uribarri *et al*, a single oral AGE challenge was associated with lower flow mediated dilation (44). Therefore, clinical studies clearly demonstrate that exogenous AGEs may have deleterious effect on vascular inflammation and endothelial dysfunction.

HMGB1 is a non histone, chromosomal protein that are released extracellularly during cell necrosis and by activated macrophage and monocytes (40). HMGB1, through its receptor RAGE, may activate vascular endothelial cells, vascular smooth muscle cells and macrophage/monocytes to upregulate proinflammatory cytokines and cell adhesion molecules. *In vivo*, prolonged elevation of HMGB1 is associated with increased lethality from sepsis in experimental sepsis (45,46), and intratracheal administration of HMGB1 is associated with increased neutrophil infiltration and acute lung injury in animal models (45,47). Taken together, HMGB1 and its interaction with RAGE play an important role for mediating inflammation response.

S100/calgranulins are a family of more than 15 myeloid related, calcium binding polypeptides that are found extracellularly at sites of inflammation. Among the S100 family of proteins, S100A12 and S100B are shown to activate proinflammatory reactions in endothelial cells, monocytes and vascular smooth muscle cells through their interaction with RAGE (16,18,21).

#### 4. ROLE OF RAGE IN ATHEROSCLEROSIS

Atherosclerosis is a chronic inflammatory disease due to multiple interaction between macrophage, T cell lymphocytes, vascular smooth muscle cells and endothelial cells (2,48). The initiation of atheroma in the large to medium arteries is triggered by the infiltration of oxidized LDL cholesterol in the intimal wall. The oxidized LDLs increases endothelial dysfunction, which may be potentiated by other risk that increase oxidative stress, such as hypertension, smoking and diabetes (2,49,50). The activated endothelium increases the expression of cell adhesion molecules that increase the uptake of monocytes into the media. The monocytes are subsequently differentiated into macrophages which take up modified LDLs, resulting in the transformation into foam cells (2,51). Also, the activated macrophages secrete free radicals and cytokines, amplifying the inflammatory cascades. The further increase in inflammation results in formation of

enough foam cells to progress fatty streaks into lipid core, with infiltration of macrophage, T cells and mast cells in the cap and shoulders of the atheroma (2,52). Cytokines secreted from the proinflammatory cells in the shoulder region increases the formation of matrix metalloproteinase and cysteine protease, which predisposes to increased risk of plaque rupture and acute coronary syndrome (2,53,54).

Recent studies have shed light on the potential role of RAGE in the pathogenesis of atherosclerosis. Activated endothelial cells, vascular smooth muscle cells in atherosclerotic plaques and activated inflammatory cells all have increased expression of RAGE, which with its interaction with RAGE ligands, increase the secretion of proinflammatory cytokines and cell adhesion molecules. Furthermore, RAGE may have a significant role in leukocyte recruitment into the intima of the atherosclerosis (27). The fact that RAGE acts to both recruit and activate macrophage suggests an important role for RAGE in the initiation and maintenance of inflammation in atherosclerosis (55).

Apo E knockout mice are models of hypercholesterolemia induced atherosclerosis that is widely used to study atherosclerosis *in vivo*. In aortas of Apo E knockout mice, there is a time dependent increase of RAGE expression, with a 6 fold increase at 24 weeks compared to baseline of 6 weeks of age (56). Apo E knockout mice were associated with accelerated atherosclerosis and increased expression of VCAM-1, MCP-1, CD-40, IL-10, MAP kinase, and MMP-2 (56). In ApoE, RAGE double knockout mice model (RAGE  $-/-$ , Apo E  $-/-$ ), the expression of the above mentioned proinflammatory mediators were significantly attenuated, suggesting the importance of RAGE signaling in propagation of vascular inflammation in atherosclerosis (56). From the above mentioned study, the expression of RAGE ligands such as S100B and HMGB1 were also attenuated in the aorta of Apo E, RAGE double knock out mice, suggesting that RAGE acts as a positive regulator of its own ligand for receptor activation (56,57).

Diabetes is a major risk factor of atherosclerosis due to its role in acceleration of atherosclerosis and vascular inflammation (57,58). One of the mechanisms for the increased risk of atherosclerosis in diabetes may be the increased accumulation of AGEs in the vascular wall (57,59). The accumulation of AGEs and its interaction of RAGE may play a significant role in the acceleration of atherosclerosis in diabetes. Diabetic Apo E knockout mice are associated with increased plaque accumulation compared to non diabetic Apo E knockout mice (57). However, diabetic RAGE, Apo E double knockout mice (RAGE  $-/-$ , Apo E  $-/-$ ) have significantly reduced plaque areas, findings that suggest that RAGE plays an important role for progression of atherosclerosis in diabetes (57). In hyperglycemia, recent study demonstrated that increased expression of RAGE and of its proinflammatory endogenous ligands (S100A8, S100A12, HMGB1) are a consequence of hyperglycemia-induced ROS (60). Thus, in diabetes, increased oxidative stress and AGE

accumulation may act in concert to increase vascular inflammation through the activation of RAGE.

### 5. RAGE AND REPERFUSION INJURY IN MYOCARDIAL INFARCTION

Despite the marked improvement in the prognosis of acute myocardial infarction via reperfusion with mechanical or thrombolytic therapy, reperfusion itself results in myocardial damage via reperfusion injury (61-64). Among the various mechanisms that have been reported to explain this phenomenon, increased oxidative stress and inflammation have been reported to be significant contributors to reperfusion injury (62). Ischemia/reperfusion injuries in rats and mice have demonstrated increased expression of RAGE in the endothelium and macrophage, which is attenuated in RAGE  $-/-$  mice (63-65). Also, reperfusion injury was associated with increase in markers of apoptosis, which was associated with significant attenuation of phosphorylation of STAT 3, a transcription factor that has been reported to confer protective effect from ischemia/reperfusion injury (65,66). Conversely, in RAGE  $-/-$  mice, the phosphorylation of STAT3 was increased with significant decrease in markers of apoptosis (65).

Increased oxidative stress is one of the major mechanisms for ischemia/reperfusion injury. Studies have shown that increase in inducible nitric oxide is associated with magnification of oxidative stress due to increase in peroxynitrite that may contribute to increased myocardial injury (63,67). In murine models of ischemia/reperfusion injury, the expression of iNOS has been demonstrated to be increased, whereas I/R injury in RAGE knockout mice model shows a significant decrease in iNOS expression (63,64).

The recruitment of leukocytes may increase myocardial damage by increasing oxidative stress, release of cytokines as well as direct capillary plugging (62). Because RAGE functions as a leukocyte adhesion molecule for mediating innate immune response, it may play a significant role in attenuating leukocyte recruitment and inflammation in the myocardium after I/R injury (27,55). Taken together, RAGE may play a significant role in mediating reperfusion injury in myocardial infarction through its activation of inflammation, increase of oxidative stress and leukocyte recruitment.

### 6. RAGE AND HYPERTENSION

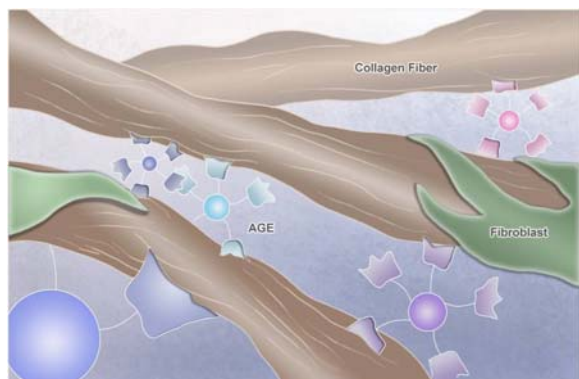
The pathophysiology of hypertension can be characterized by the increase in central aortic stiffness, which results in predominant elevation of systolic blood pressure, and increase in peripheral vascular resistance of the resistance arteriole, resulting in elevation of diastolic blood pressure (68). The increase in central aortic stiffness is usually associated with aging but may also be accelerated with diabetes (59). Previous experiments have shown that in experimental diabetes, AGEs is known to accumulate in aortic tissues and decrease collagen solubility through AGEs induced collagen cross link formation (69). In

addition to the direct effect of AGEs on aortic compliance, AGEs may theoretically interact with RAGE to increase central aortic stiffness. The activation of RAGE through AGE stimulation induces activation of p21ras, extracellular signal related kinase (ERK), p44/p42 mitogen activated protein kinase, c-Jun N-terminal kinase/stress activated kinase (JNK/SAPK), Janus kinase/signal transducer and activator of transcription (JAK/STAT), all mitogenic stimuli that induces vascular smooth muscle cell proliferation (18-26). Also RAGE induced increase in expression of cytokines, cell adhesion molecules and MMP activation may result in vascular inflammation, fibrosis and elastinolysis that is characteristic of the pathophysiology of central arterial stiffness (56). Therefore, it is highly probable that AGE accumulation associated with aging, oxidative stress and diabetes may, through its RAGE independent and dependent effect, accelerate central aortic stiffness and play an important role for the development of systolic hypertension.

In the microcirculation, the healthy endothelium is essential for maintaining adequate nitric oxide secretion for vasodilation. In hypertension, endothelial dysfunction is characterized by increased breakdown of NO breakdown, due mainly to increased oxidative stress production. RAGE activation is characterized by increased production of ROS, which may contribute to increased NO breakdown and eNOS uncoupling (70,71). Studies have shown that coronary arterioles isolated from diabetic mice with leptin resistance demonstrate impaired relaxatory response to acetylcholine and show partial improvement of the acetylcholine response after treatment with soluble RAGE (71,72). Also, the improvement of vascular tones by coinubation with NO donor sodium nitroprusside was blocked by administration of RAGE ligand S100B (71,72). The results from the above mentioned study demonstrate that RAGE may play a role in modulating vascular reactivity in the microcirculation, which may have pathophysiologic implications in hypertension.

### 7. RAGE AND AORTIC DISEASE

Abdominal aortic aneurysms are typically characterized by progressive expansion in the diameter of the infrarenal abdominal aorta that are associated with high mortality when ruptured. Although the pathogenesis of abdominal aortic aneurysms are not well known, increased vascular inflammation, oxidative stress and activation of MMP-9 activity may play a significant role. A recent study demonstrated that human S100A12 mediates the increase in interleukin-6 production, activation of transforming growth factor beta pathways and enhanced oxidative stress in primary aortic smooth muscle cell cultures from resected thoracic aortic aneurysms in S100A12 transgenic mice. They also examined S100A12 expression in aortic tissue from patients with thoracic aortic aneurysm and found increased S100A12 expression in vascular smooth muscle cells (73). Also, it has been shown *in vivo* that RAGE knockout in mouse model of abdominal aortic aneurysm is associated with significant reduction of both the incidence and diameter of the abdominal aortic aneurysm with significant reduction of the MMP-9 activity,



**Figure 2.** Cross linkage of collagens by advanced glycation endproducts will result in increased stiffening of the arteries and is a key pathogenic process in arteriosclerosis.

the activity of which was expressed abundantly in the aneurysmal wall of control (74). The increased expression of RAGE in aortic aneurysms, with subsequent increase in the RAGE ligand-RAGE interaction, may induce MMP-9 in the leukocytes, which is important for the elastin and collagen degradation that is characteristic of abdominal aortic aneurysms (74). Also, administration of soluble RAGE, a decoy receptor for RAGE ligands that attenuate the effects of RAGE activation, in the abdominal aortic aneurysm models demonstrated a significant inhibition of abdominal aortic aneurysm formation, suggesting that RAGE may potentially be a therapeutic target for inhibiting the progression of abdominal aortic aneurysms.

Both RAGE-AGEs interaction or direct collagen crosslink of AGEs (Figure 2) can cause aberrant thickening of the aortic media, disarray of elastic fibers and increased collagen deposition leading to a progressive dilatation of the aorta. In a study performed on the possible prevention of collagen crosslink of AGE by aminoguanidine, there was a significant reduction of diabetes-induced myocardial stiffness by the decreased formation of myocardial collagen AGEs (75). The development of thiazolium derivatives that catalytically break down existing glucose-derived crosslinks between proteins enables a more direct assessment of the contribution of protein crosslinking to the magnitude of age- or disease-associated changes in arterial and ventricular stiffness (76). The crosslink breaker, 3-phenacyl-4,5-dimethylthiazolium chloride (ALT-711), have been demonstrated to improves arterial and ventricular function in older rhesus monkeys (77) and vascular compliance in humans (78). In experimental diabetes, ALT-711 reverses large artery stiffness (79), whereas the crosslink breaker, *N*-phenacylthiazolium bromide, prevents vascular AGE accumulation (80).

## 8. THE POTENTIAL CLINICAL APPLICATIONS OF SOLUBLE RAGE IN CARDIOVASCULAR DISEASE

### 8.1. Structure of sRAGE

RAGE is part of the immunoglobulin superfamily and consists of three immunoglobulin-like regions, one “V”-domain followed by two “C”-domains that constitute the extracellular domain (17). RAGE also contains a single

transmembrane domain and a 43-amino acid cytosolic tail. Studies have shown that the V-domain is critical for ligand receptor binding whereas the cytosolic tail is essential for RAGE-mediated intracellular signaling. The truncated form of RAGE lacking the cytosolic tail, though competent to bind ligands, acts as a dominant negative (DN-RAGE) receptor due to the suppression of the RAGE-mediated intracellular signaling (81,82) (Figure 3).

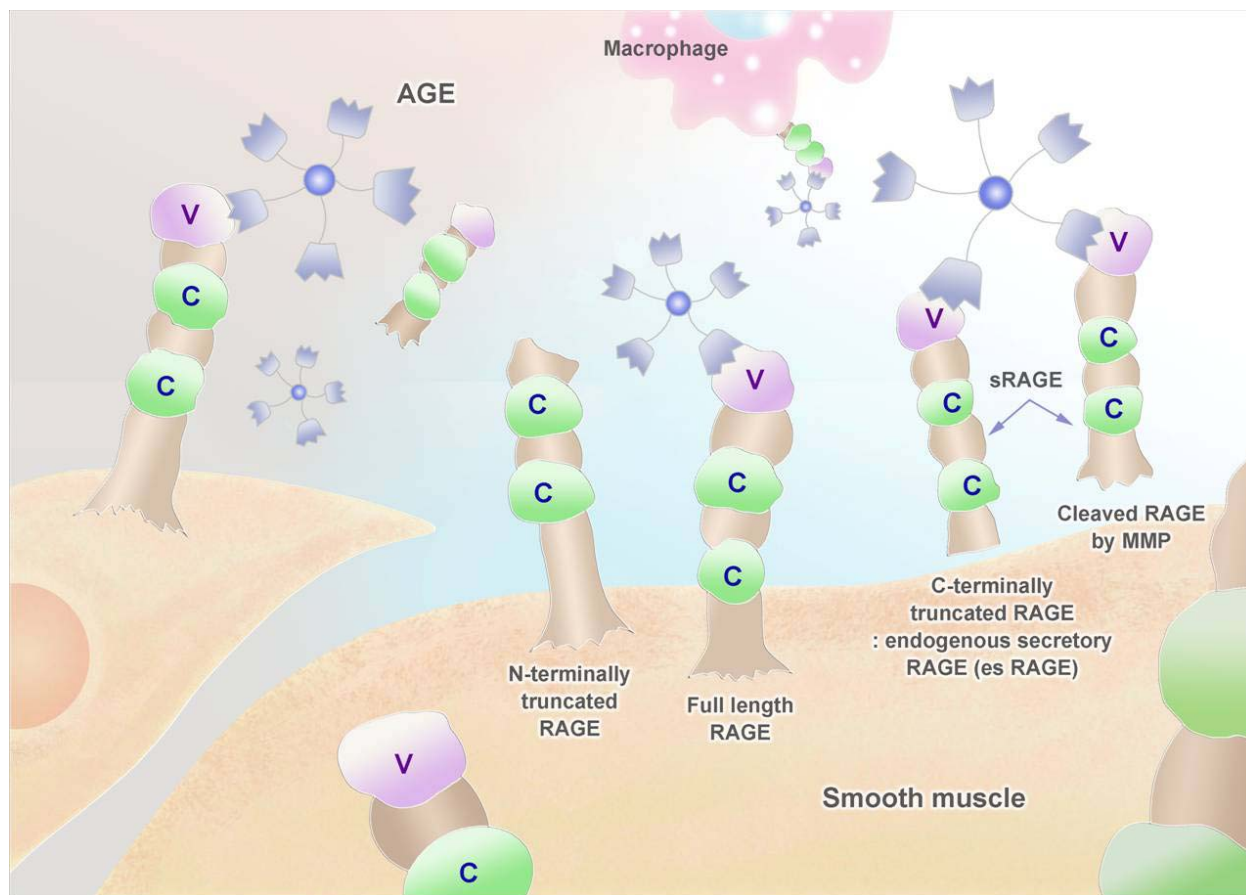
Soluble RAGE is a circulating form of RAGE that consists of V and C domains without the cytosolic and transmembrane domain. It is formed by either alternative splicing, forming the esRAGE, or by proteolytic cleavage of the transmembrane domain by metalloprotease such as ADAM10 (83). Soluble RAGE may function as a decoy receptor by binding to RAGE ligands and inhibiting RAGE ligand-RAGE interaction. Recent studies have focused on the potential role of sRAGE as a therapeutic decoy receptor protein that may attenuate vascular inflammation and atherosclerosis (58,84).

### 8.2. sRAGE as a biomarker of cardiovascular disease

There is a growing body of evidence that RAGE may play a central role in the pathogenesis of diabetic vascular complications (84,85). Moreover, RAGE expression in the vasculature is enhanced in diabetes with increased amount of sRAGE being generated from the cleavage of increased RAGE on endothelial cell surface (86,87).

Recently, there have been several studies regarding the potential role of sRAGE or esRAGE as biomarkers of RAGE ligand-RAGE activation. The reports from various studies have shown contradictory results of sRAGE being a biomarker of both increased tissue RAGE activation (88-90) and an endogenous protective factor (91,92). Falcone C *et al.* demonstrated significantly lower level of sRAGE in non-diabetic coronary artery disease patients (92) and Katakami N *et al.* demonstrated that circulating esRAGE level was significantly lower in type 1 diabetic patients with an inverse association with the severity of diabetic vascular complications (intima-media thickness, IMT) (93). The results demonstrating inverse correlation between indices of cardiovascular disease and sRAGE suggest that sRAGE may act as an endogenous protective decoy receptor to modulate RAGE activation in the vascular wall. Contrary to the above findings, Nakamura K *et al.* reported in a Japanese population that serum sRAGE levels increased in type 2 diabetic patients compared with non-diabetic control (94) and demonstrated a positive correlation of sRAGE with indices of inflammation (95). This and other contrasting reports suggest that sRAGE may be a marker of increased RAGE activation that becomes shedded into the circulation at an increased concentration during tissue RAGE activation. Since serum levels of sRAGE and esRAGE in humans are 1000–5000 times lower, respectively, than needed for the binding of AGEs, it may well be that sRAGE detected in the circulation may not be of sufficient amount to exert a biological effect as a protective decoy receptor (58,84,96-98). More evidence and data is required to determine the exact biological role of circulating sRAGE at this time.





**Figure 3.** Soluble RAGE may act as a decoy receptor by competitively inhibiting the binding of RAGE ligands to RAGE. The subsequent inhibition of RAGE ligand RAGE interaction may attenuate tissue inflammation that is associated with RAGE activation.

### 8.3. Therapeutic role of sRAGE

Several studies have shown that sRAGE is a pharmaceutically relevant therapeutic molecule with beneficial effects *in vivo* for animal models of cancer (99), chronic autoimmune inflammatory diseases (100) and diabetes (101,102). In mouse models for diabetes and atherosclerosis, depletion of RAGE gene (*ager*) results in significant attenuation of atherosclerosis with reduction in inflammatory indices (1,12). In addition to membrane-bound RAGE, *ager* also produces a minor product that lacks the membrane anchor and the cytosolic signaling domain. This product is secreted into extracellular milieu as a soluble protein (sRAGE) (1,12). Unlike RAGE that transmits signals to activate various cellular programs and causes inflammation, sRAGE functions as a natural decoy that binds RAGE ligands and prevents RAGE signaling. Studies performed in animal models of atherosclerosis demonstrated that administration of sRAGE significantly reduces vascular inflammation and attenuates the development of atherosclerosis, suggesting a therapeutic potential of sRAGE for atherosclerotic cardiovascular disease (58, 84,101).

Diabetes is a major risk factor of atherosclerosis with cardiovascular disease being the most common cause

of mortality in patients with diabetes. The elevated level of AGEs and its role in mediating oxidative stress and vascular inflammation may play a significant role for the pathogenesis of diabetes induced atherosclerosis (44,103). In streptozocin induced diabetic apo E knockout mice model, administration of sRAGE suppressed both the development of atherosclerosis and the progression of atherosclerosis (58,84). In streptozocin induced diabetic apo E knockout mice model of atherosclerosis, the intraperitoneal administration of murine sRAGE at 100ug/day for 6 weeks resulted in significant suppression of both the lesion area and lesion complexity. The immunohistochemical staining revealed that treatment with sRAGE was associated with significant decrease in the number of macrophage and smooth muscle cells. The quantitative immunohistochemical staining revealed significant decrease in the expression of mediators of inflammation such as JE-MCP-1, VCAM-1, COX-2 (84). In the clinical setting, type 2 diabetes is a more common form of diabetes and is a major underlying disease for cardiovascular disease. In type 2 diabetes model of leptin resistant, apo E knockout mice (apo E<sup>-/-</sup> db/db), there is an accelerated generation of atherosclerosis that is associated with increased expression of VCAM-1, tissue factor and MMP-9 activity in the aorta compared to non diabetic mice

(101). The administration of sRAGE in the apoE <sup>-/-</sup> db/db mice was associated with significant attenuation of atherosclerosis and inhibition of the expression of VCAM-1, tissue factor and MMP-9 activity. Also, the administration of sRAGE in non diabetic apoE <sup>-/-</sup> m/db mice resulted in significant attenuation of atherosclerosis as well (101). The results from these findings show that soluble RAGE, by competitively inhibiting the binding of RAGE ligands with RAGE, attenuates vascular inflammation and progression of atherosclerosis that are mediated by RAGE activation. The RAGE mediated vascular inflammation may be important for the pathogenesis of atherosclerosis in non diabetic conditions as well.

Neointimal formation and restenosis is one of the major limitations of percutaneous vascular intervention, which is more exaggerated in diabetes (104). The pathogenesis of neointimal formation is characterized by platelet adhesion and inflammatory cell recruitment at the site of injury, followed by exaggerated proliferation of VSMC in response to various growth factors and cytokines that are secreted by the activated platelets and leukocytes (104,105). The intraperitoneal administration of 0.5mg/day murine sRAGE in carotid artery balloon injury models of both type II diabetes rat model (Zucker obese rats) and non diabetic rats (Zucker lean rats) resulted in a significant attenuation of neointimal hyperplasia in both types of animal models. The results from this study suggest the importance of RAGE in mediating vascular inflammation during the formation of neointimal hyperplasia and the possibility for the therapeutic application of sRAGE for attenuating neointimal hyperplasia after percutaneous vascular intervention.

Although the results from the small animal studies seem promising, there are numerous hurdles to overcome before the clinical application of sRAGE for treatment of cardiovascular disease can become a reality. The doses used in these animal studies were extremely high doses for extended periods that do not seem to be applicable in large animals and human studies (84). Part of that may be due to the fact that recombinant sRAGE used in these studies were constructed and produced in non mammalian baculovirus systems which may increase the risk for endotoxin contaminations, and may lack complete biological functions due to the lack of eukaryotic post-translational modification systems that may limit protein modification and stability (106). The development of methodologies for large scale production and purification of recombinant sRAGE in mammalian cell systems is needed before sRAGE can be applicable in clinical trials.

## 9. CONCLUSION

In conclusion, initial insults resulting in endothelial dysfunction will result in leukocyte infiltration, oxidative stress and vascular inflammation that is amplified by RAGE activation. RAGE and its interaction with RAGE ligands may be important for initializing and maintaining the pathological processes that result in atherosclerosis and cardiovascular disease. Blocking RAGE ligand-RAGE interaction by administration of sRAGE results in

significant attenuation of atherosclerosis and neointimal hyperplasia in small animal models. Thus, RAGE is a promising new target for the development of novel treatments of cardiovascular disease.

## 10. ACKNOWLEDGEMENT

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