Role of Advanced Glycation End Products and Its Receptors in the Pathogenesis of Cigarette Smoke-Induced Cardiovascular Disease

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Cigarette smoking is a major risk factor for coronary artery disease (CAD), stroke, and peripheral vascular disease.1,2 Smoking predisposes to aortic and peripheral atherosclerosis resulting in abdominal aortic aneurysm and intermittent claudication.3 Epidemiological studies show that cigarette smokers have greater risk of developing CAD than nonsmokers.1–7 Coronary angiography in severe cigarette smokers has shown that the coronary arteries are atherosclerosed.8 Cigarette smoking is an independent predictor of new coronary

The interaction of advanced glycation end products (AGEs) with its cell-bound receptor RAGE increases gene expression and release of proinflammatory cytokines and increase generation of reactive oxygen species (ROS). Circulating receptors, soluble RAGE (sRAGE), and endosecretory RAGE (esRAGE) by binding with RAGE ligands have protective effects against AGE–RAGE interaction. Cigarette smoking is a risk factor for coronary artery disease, stroke, and peripheral vascular disease. This article reviews; if the AGE–RAGE axis is involved in the cigarette smoke-induced cardiovascular diseases. There are various sources of AGEs in smokers including, gas/tar of cigarette, activation of macrophages and polymorphonuclear leukocytes, uncoupling of endothelial isoform of nitric oxide synthase (eNOS) and xanthine oxidase. The levels of AGEs are elevated in smokers. Serum levels of sRAGE have been reported to be reduced, elevated, or unchanged in smokers. Mostly the levels are reduced. There is one article which shows an elevation of levels of sRAGE in smokers. Serum levels of esRAGE are unaltered in smokers. Mechanism of AGE–RAGE-induced atherosclerosis has been discussed. Atherosclerosis leads to the cardiovascular diseases. It has been suggested that ratio of AGE/sRAGE or AGE/esRAGE is useful in determining the deleterious effects of AGE–RAGE interaction in smokers. sRAGE alone is not a good marker for smoke-induced cardiovascular disease. In conclusion cigarette smoke induces formation of AGEs and reduces sRAGE resulting in the development of atherosclerosis and related coronary heart disease, stroke, and peripheral vascular disease. Ratio of AGEs/sRAGE is a better marker for cardiovascular disease than AGEs or sRAGE alone in smokers.
lesion. The mechanisms of cigarette-induced cardiovascular diseases are numerous including, atherosclerosis, thrombosis, and coronary spasm. Oxidative stress has been implicated in the development of atherosclerosis. Cigarette smoking may induce atherosclerosis through reactive oxygen species (ROS). Cigarette smoking could generate ROS through gas/tar of cigarette, activation of macrophage and polymorphonuclear leukocytes, uncoupling endothelial isoforms of NOS, and xanthine oxidase.

Advanced glycation end products (AGEs) and its receptor, soluble receptors for AGEs (sRAGE) have been implicated in the cardiovascular diseases. It has been reported that the serum levels of sRAGE are low while the levels of AGEs are high in patients with nondiabetic CAD. In animal studies it has been shown that AGE–RAGE axis is implicated in the development of atherosclerosis.

Interaction of AGEs with its cellular receptor, receptor for AGEs (RAGE) produces ROS. The sRAGE, the other receptor for AGEs acts as a decoy for RAGE ligands (AGEs) and compete with membrane-bound RAGE for ligand binding and thus reduce the generation of ROS.

It is possible that low serum levels of sRAGE and high levels of AGEs are involved in the cigarette smoke-induced cardiovascular diseases.

The present review deals with the AGE–RAGE axis, sources of AGEs in smokers, serum levels of AGEs, sRAGE and esRAGE in smokers, and mechanism of atherosclerosis.

AGEs–RAGE Axis

AGEs are heterogeneous group of irreversible adducts resulting from the nonenzymatic glycation and glycoxidation of proteins, nucleic acid, and lipids. Glucose interacts with free amino group of lysine or arginine, producing Schiff bases, which are rapidly transformed into Amadori products. A series of complex reactions results in the formation of AGEs including Nε-carboxymethyl-lysine, Nε-carboxyethyl-lysine, pentosidine, furosine, and pyraline. There are four receptors for AGEs: full length RAGE, a member of the immunoglobulin super family and is multiligand receptor; N-truncated RAGE, C-terminal RAGE having two isoforms, cRAGE (cleaved RAGE) and esRAGE. cRAGE is proteolytically cleaved from cell surface while esRAGE is alternative splicing to remove the transmembrane region. Both esRAGE and cRAGE are extracellular and functionally equivalent and interact with the same RAGE ligands. Measurement of total sRAGE includes both cRAGE and esRAGE (sRAGE enzyme-linked immunosorbent assay kit) while measurement of esRAGE measures only sRAGE which is about 20% of the total sRAGE.

Interaction of AGES with full length RAGE activates nuclear factor κappa-B (NF-κB), increases gene expression and release of inflammatory cytokines, and increases production of ROS.

Both sRAGE and esRAGE act as a decoy for RAGE by binding with RAGE ligands and preventing them from binding with membrane-bound full length RAGE. sRAGE and esRAGE have protective effects against AGE–RAGE interaction.

Sources of AGEs in Smokers

The information related to the sources of AGEs in smokers is very few. Cerami et al did an extensive study on the formation of AGEs from tobacco leaves and cigarette smoke. They reported that reactive glycation products (glycotoxins) are present in the water extracts of tobacco leaves and tobacco smoke promote formation of AGEs in vivo and in vitro. The generation of AGEs by cigarette smoke was concentration and time dependent. They also showed that cigarette smoke condensate was approximately 10-fold less active in production of AGEs than aqueous tobacco leave extracts. The AGEs-forming activity is extremely labile in both cases. The glycotoxins are highly reactive and can induce AGEs formation in hours. The AGEs formation with glucose or glucose-6-phosphate takes days to weeks. Glycotoxins from cigarette are inhaled into lung alveoli and then transformed to blood stream or lung cells where they can interact with other glycation products and contribute to the formation of AGEs.

Nicholl and Bucala proposed that certain components of cigarette smoke can interact with plasma and extracellular matrix proteins to form covalent adducts with many properties of AGEs. The data suggest that cigarette smoke can produce AGEs.

Serum Levels of AGEs

Serum levels of AGEs are elevated in cigarette smokers. Radoi et al reported that during smoking, the combustion of pre-AGE compounds present in tobacco produces a series of reactive and toxic AGE. AGE or LDL-linked AGE levels in serum are elevated in cigarette smokers. In another study, Berg et al have shown that current or past smokers have increased serum levels of AGEs compared with those who never smoked, median levels of AGEs were 10.7 U/mL (range, 7.8–16.5 U/mL) and 9.5 U/mL (range, 7.1–13.8 U/mL) (smokers vs. nonsmokers). AGEs levels are not only elevated in the serum but also in the tissue. Nicholl et al have shown that the levels of AGEs or immunologically related molecules are higher in the lenses and blood vessels of cigarette smokers. These data suggest that serum and tissue levels of AGEs are elevated in cigarette smokers.

Serum Levels of sRAGE and esRAGE in Smokers

The serum levels of sRAGE in smokers are variable. There are various reports which show a decrease in serum levels of sRAGE in smokers. Gopal et al reported that plasma levels of sRAGE were lower in ex-smokers as compared with the never smokers. In patients with chronic obstructive pulmonary disease (COPD) the sRAGE levels were not significantly different between ex-smokers and current smokers. However, Iwamoto et al reported that the plasma levels of sRAGE are lower in smokers with COPD (969.1 ± 406.0 pg/mL) and smokers without COPD (973.4 ± 426.5 pg/mL) as compared with nonsmokers (1,201.1 ± 483.6 pg/mL). Reduction of
serum sRAGE in smokers has been also reported by Yokota et al.\textsuperscript{45} On the contrary to the above reports, Biswas et al\textsuperscript{46} showed an elevated level of serum sRAGE in smokers. The levels were elevated in nondiabetic healthy cigarette smokers (1.475 ± 422 pg/mL) as compared with nonsmokers (1.165 ± 350 pg/mL). Serum sRAGE were significantly correlated with number of cigarette smoked per day. Serum sRAGE was positively correlated with the smoking habit. The levels of serum sRAGE were not significantly different between healthy control subjects and healthy smokers.\textsuperscript{47}

The serum levels of esRAGE were not significantly different between never smokers and ex-smokers, and between ex-smoker and current smokers.\textsuperscript{43} The data suggest that serum levels of sRAGE are low, high, or unaltered in cigarette smokers. The levels of esRAGE are not affected by cigarette smoke.

**Mechanism of AGEs–RAGE Axis-Induced Atherosclerosis**

Interaction of AGEs with full length RAGE increases production of ROS, activates NF-κB and increases the expression of adhesion molecules and cytokines.\textsuperscript{31,32,37} Cytokines are known to stimulate granulocytes to generate ROS.\textsuperscript{48–51}

ROS have been implicated in the development of atherosclerosis.\textsuperscript{13,52} Oxidative hypothesis of atherosclerosis has been detailed elsewhere.\textsuperscript{53} In short low-density lipoprotein (LDL) cholesterol is mildly oxidized to minimally modified LDL (MM-LDL) which stimulates smooth muscle cells and endothelial cells to produce monocyte chemoattractant protein-1 (MCP-1). ROS increases the expression of cell adhesion molecules, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and endothelial leukocyte adhesion molecules on the endothelial cells.\textsuperscript{54–57} Cell adhesion molecules help monocytes to adhere to the endothelial cell surface. MM-LDL is further oxidized to oxidized-LDL (OX-LDL). MCP-1 and OX-LDL facilitate the migration of monocyte to migrate to the subendothelial area. MM-LDL induces release of monocyte colony stimulating factor from endothelial cells that help in differentiation of monocyte/macrophage. Differentiated macrophage develops receptor for OX-LDL which is taken up by macrophage to form foam cells. Macrophage increases the expression of numerous growth factors which leads to the formation of collagen, elastic fiber, and protein and transcription of smooth muscle cells. Proliferation and migration of smooth muscle cells, synthesis of connective tissue and matrix, migration of monocyte and formation of foam cells, culminate in the initiation and progression of atherosclerosis. The role of AGEs–RAGE interaction in the development of atherosclerosis and the protective effects of sRAGE and esRAGE are depicted in Fig. 1.

Interaction of AGE and RAGE will produce atherosclerosis. sRAGE and esRAGE interacts with AGEs and hence fewer AGEs are available to interact with RAGE resulting in the reduced production of ROS, cytokines, and cell adhesion molecules. This will result in attenuation of the formation of atherosclerosis.

**Fig. 1** Figure shows the role of the interaction of AGEs and RAGE in the development of atherosclerosis and protective effects of sRAGE and esRAGE against AGE and RAGE-induced atherosclerosis. (-), decrease; ↑, increase; AGEs, advanced glycation end products; esRAGE, endogenous secretory RAGE; NF-κB, nuclear factor-kappa B; RAGE, receptor for AGEs; ROS, reactive oxygen species; sRAGE, soluble receptor for RAGE.

**Comments**

Most of the published data show that there is a reduction in the serum levels of sRAGE in cigarette smokers\textsuperscript{43–45} except one report which shows an increase in the serum levels of sRAGE in smokers.\textsuperscript{46} It is very difficult to reconcile these differences in the serum levels of RAGE in smokers. There are numerous data which suggest a protective role of sRAGE against deleterious effects of AGE–RAGE interaction\textsuperscript{22–26} in patients with nondiabetic CAD. AGE–RAGE axis has been implicated in the development of atherosclerosis in animals and sRAGE has been reported to have protective effects against atherosclerosis.\textsuperscript{28–30} As mentioned in the AGE–RAGE axis section of this review, interaction of AGE with RAGE increases the production of inflammatory cytokines, ROS and NF-κB which in turn induces proinflammatory cytokines.\textsuperscript{31,32,37} Low levels of sRAGE would increase the proinflammatory cytokines while high levels of sRAGE would reduce the proinflammatory cytokines. Biswas et al\textsuperscript{46} however reports that serum levels of sRAGE are elevated in smokers. They suggest that elevated levels of sRAGE would increase proinflammatory biomarkers and are responsible for cardiovascular disease in smokers.

Other investigators\textsuperscript{38,39} besides Biswas et al\textsuperscript{46} have suggested sRAGE a proinflammatory biomarker. Nakamura et al\textsuperscript{38} have shown that there is significant positive correlation between sRAGE and inflammatory markers in patients with type 2 diabetes. Pullerits et al\textsuperscript{39} have reported that sRAGE may be a proinflammatory and chemotactic molecule through its interaction with β 2 integrin Mac-1. However, these increases in the sRAGE may not be the culprit and proinflammatory biomarkers. These investigators did not measure the serum levels of AGEs in the studies.

Four players including, AGEs, cellular receptor RAGE, and circulating receptors sRAGE and esRAGE are involved in the AGE–RAGE axis. In humans it is not feasible to measure cell-bound receptor RAGE.
AGEs, sRAGE, and esRAGE can be measured in the serum of humans. Other players such as AGES besides sRAGE and esRAGE should be considered in the equation of biomarker for disease associated with AGE–RAGE axis. Increases in serum sRAGE may be associated with increase in serum AGES. If this occurs then increases in sRAGE may not be proinflammatory. If the increase in AGES is more than sRAGE, more RAGE is available to interact with cell-bound RAGE to produce inflammatory mediators and ROS. For example, the levels of sRAGE are elevated in diabetes and end-stage renal disease. The levels of AGES are also elevated in diabetes and end-stage renal disease. In this context, the authors would like to point out that the serum levels of AGES are elevated in smokers. It is possible that elevated levels of serum sRAGE are not enough to handle large amount of AGES. It would be scientifically sound to use the ratio of AGES/sRAGE or AGES/esRAGE as a biomarker of the disease. The statement of Biswas et al., that sRAGE is proinflammatory, does not seem scientifically sound. In this respect Prasad et al. has reported that AGES/sRAGE or AGES/esRAGE would be a better biomarker than sRAGE or esRAGE for disease associated with AGE–RAGE axis.

In conclusion serum levels of sRAGE are low and the levels of AGE are elevated in cigarette smokers. The decrease in the serum levels of sRAGE and increase in the levels of AGES may be involved in the cigarette smoke-induced cardiovascular disease in cigarette smokers. AGES/sRAGE would be better proinflammatory marker than sRAGE or AGES alone.

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