

AGE-RAGE Axis in the Pathophysiology of Chronic Lower Limb Ischemia and a Novel Strategy for Its Treatment

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Abstract

This review focuses on the role of advanced glycation end products (AGEs) and its cell receptor (RAGE) and soluble receptor (sRAGE) in the pathogenesis of chronic lower limb ischemia (CLLI) and its treatment. CLLI is associated with atherosclerosis in lower limb arteries. AGE-RAGE axis which comprises of AGE, RAGE, and sRAGE has been implicated in atherosclerosis and restenosis. It may be involved in atherosclerosis of lower limb resulting in CLLI. Serum and tissue levels of AGE, and expression of RAGE are elevated, and the serum levels of sRAGE are decreased in CLLI. It is known that AGE, and AGE-RAGE interaction increase the generation of various atherogenic factors including reactive oxygen species, nuclear factor-kappa B, cell adhesion molecules, cytokines, monocyte chemoattractant protein-1, granulocyte macrophage-colony stimulating factor, and growth factors. sRAGE acts as antiatherogenic factor because it reduces the generation of AGE-RAGE-induced atherogenic factors. Treatment of CLLI should be targeted at lowering AGE levels through reduction of dietary intake of AGE, prevention of AGE formation and degradation of AGE, suppression of RAGE expression, blockade of AGE-RAGE binding, elevation of sRAGE by upregulating sRAGE expression, and exogenous administration of sRAGE, and use of antioxidants. In conclusion, AGE-RAGE stress defined as a shift in the balance between stressors (AGE, RAGE) and antistressor (sRAGE) in favor of stressors, initiates the development of atherosclerosis resulting in CLLI. Treatment modalities would include reduction of AGE levels and RAGE expression, RAGE blocker, elevation of sRAGE, and antioxidants for prevention, regression, and slowing of progression of CLLI.

Keywords

- ▶ advanced glycation end products (AGE)
- ▶ cell receptor for AGE
- ▶ soluble receptor for AGE
- ▶ AGE-RAGE stress
- ▶ chronic lower limb ischemia
- ▶ atherosclerosis
- ▶ treatment of chronic lower limb ischemia

Lower limb ischemia is of two types: acute and chronic. Acute lower limb ischemia is mostly due to sudden obstruction of lower limb arteries because of embolus or thrombosis.¹ Very rarely aortic dissection and thrombosis of popliteal artery aneurysm may cause acute lower limb ischemia. Chronic lower limb ischemia (CLLI), a slowly progressive disease due to obliteration of arteries in the lower limb, is classified as mild, moderate, and critical.² Mild CLLI is asymptomatic. Moderate CLLI causes intermittent claudica-

tion, while critical CLLI is characterized by pain at rest and may culminate into nonhealing chronic leg ulcer and gangrene. Critical lower limb ischemia (CLI) is caused by atherosclerosis and the risk factors for CLI are similar to those of atherosclerosis, such as diabetes, dyslipidemia, smoking, hypertension, obesity, and infection.³

Prevalence of CLLI rises with age. It is uncommon before the age of 50 years but rises to 20% at the age of 80 years.⁴ It is estimated that more than 200 million people suffer from CLLI

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worldwide.⁵ The overall prevalence and incidence of CLLI in elderly people in U.S. are 0.23 and 0.20%, respectively, and increase with diabetes and aging, and are higher in male than female.⁶ It is also reported that in diabetics the risk of critical lower limb ischemia is increased by 7.6-fold. The incidence of CLLI in black Americans is 0.41% while that in white Americans it is 0.18%.

Advanced glycation end products (AGEs) and its cell-bound receptor RAGE (receptor for AGE) and soluble receptor for AGE (sRAGE) have been implicated in carotid artery de-endothelialization-induced neointima expansion in wild-type mice,^{7,8} streptozocin-induced diabetes accelerated atherosclerosis in apolipoprotein E (apoE)-deficient mice,⁹ coronary artery disease (CAD),¹⁰ and restenosis following percutaneous coronary intervention (PCI).¹¹ AGE-RAGE axis comprise of AGE, RAGE, and sRAGE. Possibilities exist that AGE-RAGE axis is involved in the pathogenesis of atherosclerosis in the arteries supplying the lower limb resulting in CLLI. If AGE-RAGE is involved in the development of chronic limb ischemia, then the levels of AGE in artery and serum, and expression of RAGE in artery will be elevated, and serum levels of sRAGE will be reduced in patients with chronic limb ischemia. Understanding the role of AGE-RAGE axis in atherosclerosis in CLLI a novel strategy can be developed for the prevention, slowing of progression, and regression of CLLI. This article gives a brief review of AGE-RAGE axis, serum levels of AGE and sRAGE, and tissue levels of RAGE expression, AGE-RAGE interaction, mechanisms by which AGE-RAGE axis induces atherosclerosis, and targeting AGE-RAGE axis for the treatment of CLLI.

AGE-RAGE Axis

AGEs are heterogeneous groups of irreversible adducts formed from nonenzymatic interaction of amino groups of proteins, lipids, and nucleic acids with reducing monosaccharides such as glucose, fructose, and glyceraldehyde.^{12,13} AGE interacts with RAGE, sRAGE, cRAGE, and esRAGE. RAGE is cell-bound receptor for AGE. There are two isoforms of RAGE: cleaved RAGE (cRAGE) which is proteolytically cleaved from full-length RAGE,¹⁴ and endogenous secretory RAGE (esRAGE) which is produced from splicing of full-length RAGE messenger ribonucleic acid (mRNA).¹⁵ sRAGE is composed of both cRAGE and esRAGE, and AGE interacts with its cell receptor (RAGE) to produce reactive oxygen species (ROS) through activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase¹⁶ which activates nuclear factor-kappa B (NF- κ B).¹⁷ Activated NF- κ B activates numerous genes like tumor necrosis factor- α (TNF- α), interleukin (IL)-1, IL-2, IL-6, IL-8, and IL-9.^{18,19} Proinflammatory cytokines upregulate NADPH oxidase²⁰ and increase the generation of ROS.²¹ sRAGE acts as a decoy for RAGE by binding with RAGE ligands.²² Binding of sRAGE with ligands does not activate intracellular signaling. sRAGE also is a competitive inhibitor of ligand-RAGE interaction.²³ AGE-RAGE stress has been defined as a shift in the balance between stressors (AGE, RAGE) and antistressors (sRAGE) in favor of stressors.²⁴

Serum and Tissue Levels of AGE in Chronic Lower Limb Ischemia

The levels of AGE have been investigated in both animals and humans.

Animals

AGE levels were elevated in blood vessels of femoral artery ligation-induced ischemic lower limb ischemia both in diabetic and nondiabetic mice.²⁵ Tamarat et al²⁶ have reported that plasma levels of AGE are markedly elevated in lower limb ischemia due to femoral artery ligation in streptozotocin-induced diabetic mice.

Humans

Skin autofluorescence (SAF) is a noninvasive measurement of levels of AGE in skin.²⁷ SAF levels are elevated in patients with peripheral artery disease (PAD).²⁸ SAF is an independent predictor of amputation of critical limb ischemia and is associated with 5-year mortality in patients with PAD.²⁹ It has been reported that SAF is elevated in patients with carotid artery stenosis as compared with control, and the levels of SAF was greater in patients of carotid stenosis with peripheral artery occlusive disease than in patients with carotid artery stenosis alone.³⁰ Nin et al³¹ in a 12-year follow-up study have reported that the levels of N(6)-carboxymethyl-lysine (CML), N(6)-carboxyethyl-lysine (CEL), and pentosidine are elevated in type 1 diabetes with cardiovascular complications including amputation because of lower limb ischemia and vascular surgery for peripheral atherosclerotic disease. These investigators also reported that the elevated levels of AGEs were associated with incidental fatal and nonfatal cardiovascular diseases and all-cause mortality independent of traditional cardiovascular risk factors. Plasma levels of AGEs and pentosidine are elevated in type 2 diabetic patients with CLLI and were inversely associated with ankle-brachial index.³² Plasma levels of AGE were elevated in patients with peripheral arterial disease.³³ Prasad et al³⁴ measured the serum levels of total AGEs (CML, CEL, methylglyoxal dimer, and pyrillamine) using AGE enzyme-linked immunosorbent assay (ELISA) kit, and CML using anti-CML specific monoclonal antibody-based ELISA kit in 16 patients with critical limb ischemia and 26 patients with lifestyle-limiting claudication. They observed that both the total AGE and CML levels were elevated in critical limb ischemia; however, the correlation was greater with total AGE than with CML. In summary, serum and tissue levels of AGEs are elevated in patients with CLLI.

Expression of RAGE in Vessels of Chronic Lower Limb Ischemia

Expression of RAGE in ischemic lower limb has been reported both in animals and humans.

Animals

Using single-photon emission computerized tomography/computed tomography scan, ex vivo gamma counting and immunohistopathology, Tekabe et al³⁵ have shown that the

expression of RAGE were higher in ischemic limb of diabetic and nondiabetic mice than in nonischemic diabetic and nondiabetic mice. Lower limb ischemia in the mice was produced by ligating left femoral artery. Streptozotocin was used to produce diabetes.

Humans

Ritthaler et al³⁶ have observed that RAGE expression in endothelial cells was elevated in patients with PAD with or without diabetes compared with healthy subjects. All selected patients were in stages IIb–IV according to Fontaine criteria for peripheral occlusive vascular disease. The control subjects had no diabetes or peripheral vascular occlusive disease. Malmstedt et al³³ reported that RAGE expression was observed in vein used for bypass in patients with peripheral arterial disease.

Serum/Plasma Levels of sRAGE in Chronic Lower Limb Ischemia

There are very few papers available in literature on the serum/plasma levels of sRAGE in CLLI in animals and humans.

Animals

Kim et al³⁷ have shown that blood flow ratio of ischemic/control limb in hindlimb ischemia model of mice was significantly reduced in diabetic mice compared with nondiabetic mice postoperatively. However, the blood flow ratio of ischemic/control was significantly greater in mice treated with sRAGE compared with control group postoperatively. They also showed that the capillary density was reduced in ischemic limb of diabetic mice compared with ischemic limb of nondiabetic mice. However, the capillary density was greater in mice treated with sRAGE compared with untreated diabetic mice. These data suggest that sRAGE levels may be lower in ischemic lower limb.

Humans

Falcone et al³⁸ measured the plasma levels of sRAGE in CAD patients with and without PAD (chronic limb ischemia). The patients with PAD had ankle-brachial index of < 0.9 and were in stage of IIb and stage III according to Fontaine classification. These authors reported that plasma sRAGE levels were 48% lower in CAD patients with or without PAD as compared with controls. sRAGE levels were 19.7% lower in CAD patients with PAD as compared with CAD patients without PAD. These values suggest that plasma levels of sRAGE are lower in patients with PAD as compared with controls.

In summary, serum/plasma levels of AGE and expression of RAGE are elevated while plasma levels of sRAGE are reduced in patients with CLLI.

Role of AGE-RAGE Axis in the Development of Atherosclerosis

AGE could induce development of atherosclerosis through nonreceptor and receptor-dependent mechanisms.

Nonreceptor-Dependent Mechanism of AGE in Development of Atherosclerosis

There are various pharmacological effects of AGE in inducing atherosclerosis. AGE modifies apoB100 which makes low-density lipoprotein (LDL) cholesterol more atherogenic.³⁹ AGE increases synthesis of extracellular matrix,⁴⁰ traps endothelial LDL,⁴¹ and cross-binds with collagen.⁴² Glycation of apoB and phospholipid component of LDL alters LDL clearance and increases the susceptibility of LDL oxidation.^{43,44} AGE increases susceptibility of LDL to oxidation.⁴⁵ AGE decreases the production of nitric oxide (NO).⁴⁶ Oxidized LDL (Ox-LDL) decreases the production of NO through reduction of NO-synthase (NOS).⁴⁷ It has been reported that AGE quenches NO.⁴⁸ Matrix-bound AGE reduces NO production,⁴⁹ decreases the half-life of NOS,⁵⁰ quenches and inactivates NO,⁵¹ and inhibits the antiproliferative activity of NO.⁵² AGE reduces NOS mRNA and protein resulting in reduction in NO levels.⁵³ Matrix-bound AGE also increases the expression of endothelin-1⁵⁴ which has been implicated in the development of atherosclerosis.⁴⁰ Glycation of LDL decreases its recognition by LDL receptors.⁵⁵ Glycated LDL increases the smooth muscle cell proliferation and differentiation.⁵⁶ AGE interferes reverse cholesterol transport⁵⁷ which will increase the extracellular cholesterol. AGE increases accumulation of cholesterol and its esters in macrophages in vitro.⁵⁸

Receptor-Mediated Mechanism

AGE interacts with RAGE to generate ROS through activation of NADPH oxidase.¹⁶ ROS then activates NF- κ B which in turn results in transcriptional activation of variety of inflammatory genes such as TNF- α , TNF- β , IL-1, IL-6, IL-8, and interferon- γ .^{18,59-61} NF- κ B induces gene for NADPH oxidase in polymorphonuclear leukocytes⁶² which would generate ROS. Interaction of AGE and RAGE enhances expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1 (VCAM-1), and E-selectin through NF- κ B.⁶⁰ In apo-deficient mice, streptozotocin-induced diabetes was associated with increased expression of VCAM-1 and tissue factor.⁶³ AGE upregulates expression of monocyte chemoattractant protein-1 (MCP-1) mRNA in mesangial cells.⁶⁴ AGE increases expression of MCP-1 and vascular endothelial growth factor in human-cultured mesangial cells.⁶⁵ AGE induces expression of MCP-1 in podocytes through activation of RAGE and generation of intracellular ROS.⁶⁶ AGE increases the expression and secretion of granulocyte macrophage-colony stimulating factor (GM-CSF) by macrophages.⁶⁷ Interaction of AGE with RAGE on mononuclear leukocytes produces phenotype of activated macrophages that promotes induction of insulin-like growth factor (IGF), IGF-1, and platelet-derived growth factor (PDGF).^{68,69} Binding of AGE with RAGE bearing mononuclear phagocytes enhances chemotaxis leading to mononuclear monocytes infiltration through intact endothelial surface.^{70,71} AGE-RAGE binding in smooth muscle cells enhances chemotactic migration, cellular proliferation, and production of fibrogen.^{72,73} AGE increases expression of transforming growth factor- β (TGF- β) which is involved in extracellular matrix formation.⁷⁴ It is to note that ROS is involved in expression of VCAMs through activation of NF- κ B. AGE-RAGE interaction

Atherogenic effects of AGE- RAGE interaction and antiatherogenic effects of AGE- sRAGE interaction

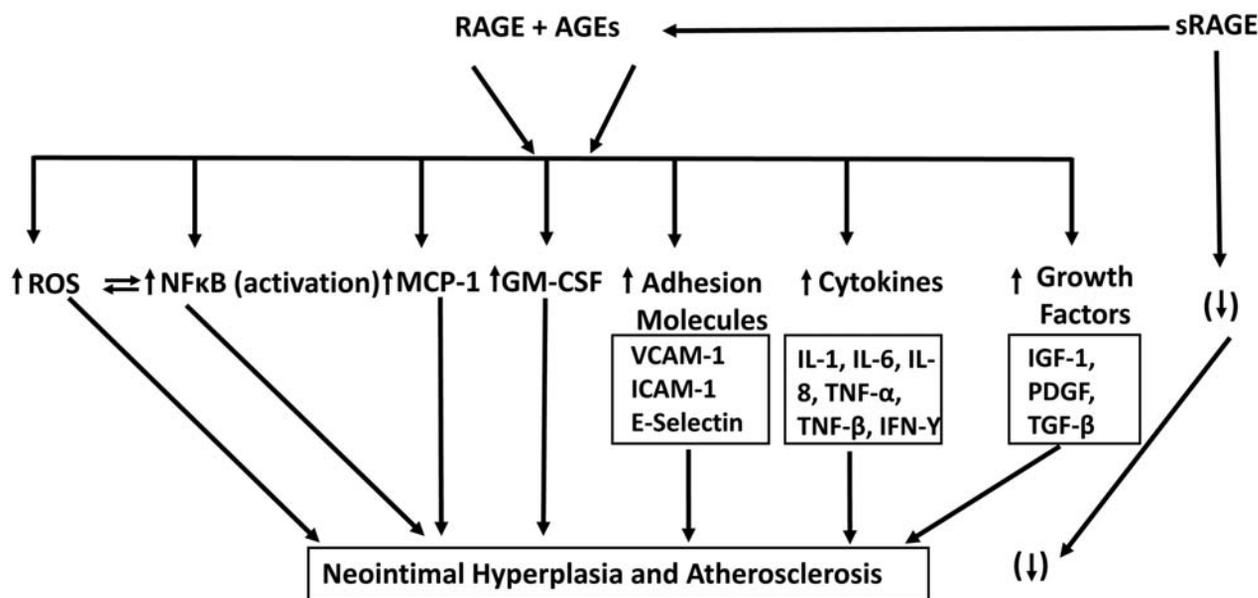


Fig. 1 Effects of interaction of AGE with RAGE and AGE with sRAGE on generation of atherogenic factors. AGE-RAGE interaction increase the generation of ROS (reactive oxygen species), NF- κ B (nuclear factor-kappa B) activation, MCP-1 (monocyte chemoattractant protein-1), GM-CSF (granulocyte macrophage-colony stimulating factor), VCAM-1 (vascular cell adhesion molecule-1), ICAM-1 (intercellular adhesion molecule -1), E-selectin; IL (interleukin), TNF- α (tumor necrosis factor- α), TNF- β , IFN- γ (interferon-gamma), which are involved in the development of neointimal hyperplasia and atherosclerosis. Interaction of AGE with sRAGE reduces the amount of AGE to interact with RAGE leading to reduction in formation of atherogenic factors resulting in decreased development of neointimal hyperplasia and atherosclerosis. AGE, advanced glycation end products; RAGE, cell receptor for AGE; sRAGE, soluble receptor for AGE; PDGF, platelet-derived growth factor; IGF-1, insulin like growth factor-1; TGF- β , transforming growth factor- β ; \uparrow , increase; \downarrow , decrease.

decreases endothelial barrier function and hence increases permeability of endothelial cell layer.^{75,76} AGE also interacts with sRAGE and has protective effect against adverse effects of AGE-RAGE interaction.^{22,23} **Fig. 1** shows the receptor-mediated effects of AGE in the development of atherosclerosis. AGE-RAGE interaction produces atherosclerosis through generation of various chemicals while sRAGE prevent the development of atherosclerosis by reducing the formation of these chemicals.

Mechanism of AGE-RAGE Axis-Induced Atherosclerosis

The proposed mechanism of AGE-RAGE-induced atherosclerosis is depicted in **Fig. 2**. It is based on oxidative hypothesis of atherosclerosis.⁷⁵ The first step in the development of atherosclerosis is mild oxidation of LDL called minimally modified LDL (MM-LDL). MM-LDL is further oxidized to produce Ox-LDL. Monocytes adhere to endothelium and transmigrate into subendothelial space.⁷⁶ Smooth muscle cells and endothelial cells exposed to MM-LDL produce MCP-1 which assist monocyte migration. Also, Ox-LDL directly enhances monocyte migration. Monocytes/macrophages express LDL receptor but the uptake of native LDL is not sufficient to produce foam cells. Ox-LDL is a ligand for scavenger receptor that is expressed when monocytes differentiate into tissue macrophages. Monocyte/macrophage differentiation is enhanced by release of monocyte-CSF from endothelial

cells stimulated by MM-LDL. Tissue macrophage has receptors for Ox-LDL to produce foam cells, a major component of fatty streak. It is an early stage of atherosclerosis. Macrophages generate numerous growth regulating molecules.⁷⁷ As mentioned above, AGE-RAGE interaction induces numerous growth factors (PDGF, IGF-1, TGF- β)⁶⁸⁻⁷⁴ which would increase smooth muscle proliferation and migration, and fibrous tissue formation. Fatty streaks develop into full-fledged atherosclerosis which is associated with smooth muscle cell and lipid accumulation, necrotic core, and formation of fibrous cap.^{72,73,77}

ROS and Atherosclerosis

Oxygen radicals have been implicated in the development of atherosclerosis.^{78,79} ROS generated by interaction of AGE with RAGE, therefore, would induce atherosclerosis.

Treatment Modalities

Considering the role of AGE-RAGE axis in the pathophysiology of atherosclerosis and hence CLLI, the treatment of CLLI should be directed toward reduction in levels of AGE, ROS, and RAGE, blocking of RAGE binding with AGE, degradation of AGE in vivo, elevation of sRAGE levels, and antioxidant (**Fig. 3**). Targeting these pathways would prevent, regress, or slow the progression of CLLI. A brief description of these targets is being described here.

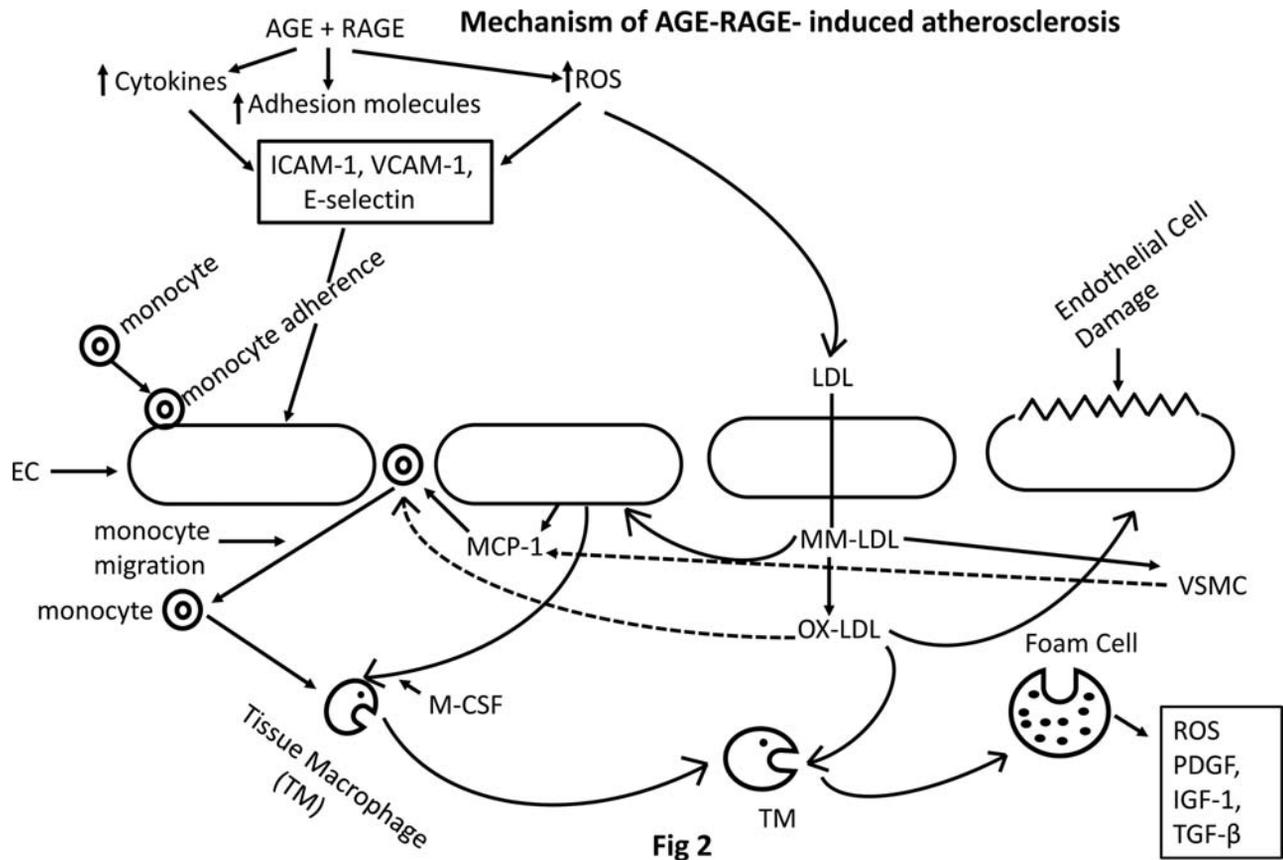


Fig. 2 Mechanism of AGE-RAGE-induced atherosclerosis. AGE, advanced glycation end products; RAGE, cell receptor for AGE; ROS, reactive oxygen species; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; LDL, low-density lipoprotein; MM-LDL, minimally modified LDL; Ox-LDL, oxidized-LDL; MCP-1, monocyte chemoattractant protein-1; M-CSF, monocyte colony stimulating factor; VSMC, vascular smooth muscle cell; EC, endothelial cell; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor-β; IL, interleukin; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-gamma; TM, tissue macrophage; EC, endothelial cell; VSMC, vascular smooth muscle cell; TM, tissue macrophage; ↑, increase.

Potential treatment targets for CLLI

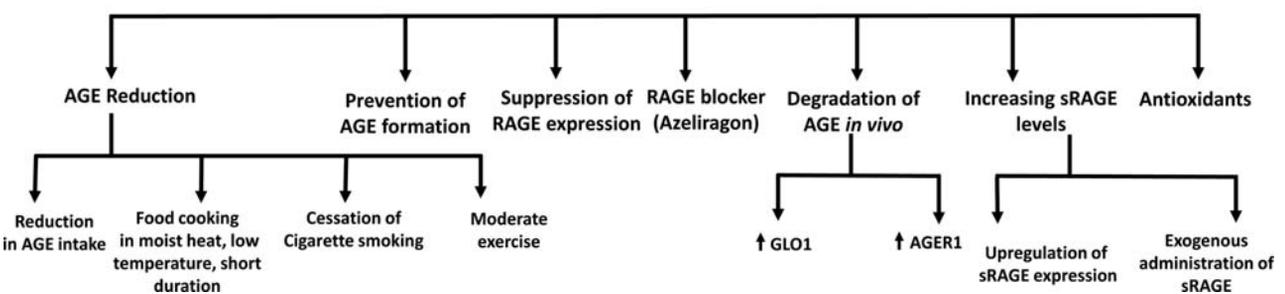


Fig. 3 Potential treatment targets for chronic lower limb ischemia (CLLI). AGE, advanced glycation end products; RAGE, receptor for AGE; sRAGE, soluble receptor for AGE; GLO1, glyoxalase 1; AGER1, advanced glycation end products receptor 1.

AGE Reduction

Reduction of AGE levels in the body can be achieved by reduction in dietary intake of AGEs, prevention of AGE formation, and degradation of AGE in the body.

AGE Intake Reduction

There are certain diets which are rich in AGE content (red meat, cheese, cream, animal fat, sweetened pastry).⁸⁰ Butter, cheese, cream, margarine, and mayonnaise contain high

quantity of AGE than oil and nuts.⁸¹ Beef has highest amount of AGE followed by poultry, pork, fish, and eggs in the meat class.⁸¹ Grains, legumes, breads, vegetables, fruits, and milk contains lowest amount of AGE.⁸¹ Fat-free milk has lower amount of AGE compared with whole milk.⁸¹ Consumption of food containing high amount of AGE should be reduced in patients with CLLI. Patients should be advised to consume less sugar because sugars participate in generation of AGEs.^{12,13} It has been reported that serum levels of AGE is

markedly reduced in healthy or diabetic individual with short-term consumption of low AGE containing diet.⁸²

Food Cooking

Cooking at high temperature in dry heat (frying, broiling, grilling, roasting) increases AGE formation more than cooking in moist heat (poaching, stewing, steaming, and boiling).⁸³ Duration of cooking also affects the formation of AGEs. The formation of AGEs is markedly reduced when cooking at low temperature in moist heat for short duration.⁸¹

Other AGE-Lowering Maneuvers

Cigarette smoking increases the serum levels of AGEs,⁸³ and hence the patient should be advised to stop cigarette smoking. AGE levels are reduced by 41 to 60% with long runs in untrained and trained subjects.⁸⁴ Reduction in serum levels of AGEs is greater with regular moderate exercise than irregular severe exercise.⁸⁵ Serum levels of AGE are reduced with Tai chi exercise of moderate intensity.⁸⁶ Patients should be advised to do regular exercise of moderate intensity.

Prevention of AGE Formation

Consumption of acidic ingredients (lemon juice, vinegar)⁸⁷ and pomegranate and its phenolic components⁸⁸ should be advocated because they prevent AGE formation. Some vitamins such as benfotiamine,⁸⁹ pyridoxine,⁹⁰ vitamin C,⁹¹ vitamin D,⁹² and vitamin E⁹³ prevent the formation of AGEs. Carnosine, an antioxidant from meat, inhibits AGE formation.⁹⁴ D-carnosine has been reported to prevent development of atherosclerosis in diabetic mice.^{95,96} Linolenic acid prevents the formation of AGEs.⁹⁷ Aminoguanidine inhibits AGE formation.⁹⁸ Clinical trials with aminoguanidine have been terminated due to its undesirable side effects.⁹⁹ Alpha-lipoic acid,¹⁰⁰ aspirin,¹⁰¹ metformin,¹⁰² pentoxifylline,¹⁰³ resveratrol,¹⁰⁴ and curcumin¹⁰⁵ are potential inhibitors of AGE formation.

Suppression of RAGE Expressions and RAGE Blockers

Statins (simvastatin, atorvastatin),^{106,107} angiotensin-II receptor blockers (candesartan, telmisartan),^{108,109} calcium channel blocker (nifedipine),¹¹⁰ antidiabetic agents (pioglitazone, rosiglitazone),¹¹¹ and curcumin¹¹² downregulate the expression of RAGE. Azeliragon (TTP488) inhibits interaction of RAGE with AGE and other RAGE ligands.¹¹³ RAGE receptor blockers have been described in detail by Bongarzone et al.¹¹⁴ Preclinical studies in animal model of Alzheimer's disease have shown that azeliragon decreases plaque deposition and slow cognitive decline.¹¹⁵ Azeliragon in low doses improves cognitive function in patients with Alzheimer's disease.¹¹³ Azeliragon treatment in mild to moderate Alzheimer's disease was found to be effective but phase 2 clinical trial was stopped because of adverse side effects. The search for RAGE blocker is on.¹¹⁶

Degradation of AGE In Vivo

Increasing Expression and Activity of Endogenous Glyoxalase 1

Glyoxalase 1 (GLO1) degrades AGE through degrading reactive dicarbonyls prior to formation of AGE.¹¹⁷ Overexpres-

sion of GLO1 would be helpful in reducing the levels AGE. It has been reported that combined use of transresveratrol found in grapes and hesperetin found in orange increased the expression and activity of GLO1 in a placebo-controlled crossover clinical trial.¹¹⁸ It was also reported that overexpression of GLO1 in lens and retinal capillary pericytes protected against hyperglycemia-induced protein modification¹¹⁹ and apoptosis.¹²⁰

Increasing Expression and Activity of Endogenous Advanced Glycation End Products Receptor 1

Advanced glycation end products receptor 1 (AGER1) degrades AGE intracellularly and is a blocker of AGE-RAGE-mediated formation of ROS and proinflammatory cytokines.^{121,122} There is no specific drug which can increase the expression of AGER1. AGER1 counteracts AGE-induced oxidative stress through inhibition of RAGE signaling.^{121,122} Since AGER1 and RAGE competes for with AGE, low concentration of AGER1 would increase the binding of AGE with RAGE, resulting in increased oxidative stress and proinflammatory cytokines. AGER1 is an AGE receptor.¹²³ Reduction in AGER1 expression is associated with elevated levels of sRAGE.¹²⁴ AGER1 is positively correlated with sRAGE in complicated diabetes.¹²⁴ The above data suggests that AGER1 may serve as a future target for treatment of CLI.

Increasing the Levels of sRAGE

Levels of sRAGE could be increased in two ways: upregulation of expression of sRAGE and exogenous administration of sRAGE.

Upregulation of sRAGE Expression

Statins such as pitavastatin and pravastatin increased the serum levels of sRAGE and reduced the vascular remodeling and atheroma in patients with CAD.¹²⁵ sRAGE levels are elevated in serum of patients with type 2 diabetes with atorvastatin.¹²⁶ Other statins (atorvastatin, fluvastatin, lovastatin) increased the sRAGE levels in isolated cell culture.¹²⁷

Angiotensin-converting enzyme inhibitors such as ramipril and perindopril increase the serum levels of sRAGE. It has been shown that ramipril increased the serum levels of sRAGE in rat,¹²⁸ and perindopril increased the serum levels sRAGE in type 1 diabetic patients.¹²⁸ Rosiglitazone¹²⁹ an antidiabetic drug elevates the serum levels of sRAGE in type 2 diabetic patients. Serum levels of sRAGE are elevated in women with polycystic ovarian syndrome with rosiglitazone.¹³⁰

Exogenous Administration of sRAGE

Animal studies suggest that AGE-RAGE axis is involved in the development of atherosclerosis. It has been reported that AGE and RAGE levels are elevated in the wall of carotid artery in Zucker diabetic rats as compared with euglycemic control rats.⁷ These authors also showed that balloon injury in carotid artery of these rats further increased the levels of AGE and RAGE in the carotid artery associated with neointimal hyperplasia. Administration of sRAGE before and up to 21 days after balloon injury significantly reduced the neointimal growth. Similarly, other investigators⁸ showed

that arterial de-endothelialization in wild-type mice increased the levels of AGE and RAGE in the injured artery and this was associated with expansion of neointima. Administration of sRAGE reduced the neointimal expansion and decreased smooth muscle cell proliferation and migration, and extracellular matrix proteins expression. Administration of sRAGE has been demonstrated to completely suppress atherosclerosis in diabetic apoE-deficient mice and this effect was independent of hyperglycemia and lipid concentration.¹³¹ sRAGE has been shown to protect ischemic stroke in animal model.¹³² The above data suggest that sRAGE could be effective in the prevention and treatment of atherosclerosis. However, no clinical trial with sRAGE has been made in human atherosclerosis as yet.

Antioxidants

As mentioned earlier, AGE-RAGE interaction produces ROS,^{16,62} which have been implicated in the development of atherosclerosis.^{78,133-135} Considering that antioxidants may be helpful in the treatment of CLLI, there are quite a few antioxidants that can be used. Antioxidants have been shown to reduce the development of atherosclerosis.^{136,137} Prasad and Kalra¹³⁸ have reported that vitamin E significantly prevented the development of hypercholesterolemia-induced atherosclerosis in rabbits. Hypercholesterolemia increases generation of ROS through various mechanisms.¹³⁸ Long-term (18 months) use of vitamin E (50 IU in diet) with low fat/cholesterol diet reduced atherosclerosis in *Ldlr*^{-/-} mice.¹³⁹ Clinical trials in humans showed some positive benefits.¹⁴⁰ Meta-analysis did not show evidence of antiatherosclerotic effects of vitamin E.¹⁴¹ Failure of antioxidant strategies may be due to inappropriate doses, lack of combination of antioxidants, application of antioxidants in very advanced atherosclerosis, and frequency of drug administration. Vitamin alone may not be effective because when vitamin E scavenges ROS, it gets converted into tocopheryl radical which is harmful. Vitamin C regenerates vitamin E from tocopheryl.¹⁴² Combination of vitamin E with vitamin C would be helpful. Vitamin C is a water-soluble antioxidant.¹⁴³

Coenzyme Q¹⁰ is a lipophilic antioxidant, scavenges peroxy radicals, and has antiatherogenic effects.¹⁴⁴ Probucol is a lipid-soluble antioxidant¹⁴³ and has antiatherogenic effect.¹⁴⁵ Prasad et al¹³⁵ have reported that probucol ameliorated the development of atherosclerosis in hypercholesterolemic rabbit. It has been shown to reduce restenosis following PCI.¹⁴⁶ It inhibits smooth muscle cell proliferation and cell adhesion molecule expression on endothelial cells.¹⁴³ There is a synthetic antioxidant BO-653n which is an analogue of α -tocopherol and inhibits development of atherosclerosis.¹⁴⁷ It reduces α -tocopheroxyl radical and inhibits LDL oxidation in the intimal area.¹⁴⁸ Other antioxidants such as garlic¹⁴⁹ and secoisolariciresinol diglucoside have been reported to prevent hypercholesterolemic atherosclerosis.¹³⁴

Perspectives

CLLI is due to atherosclerosis in the arteries of lower limb. As described earlier in this article, AGE-RAGE axis plays a role in the development of atherosclerosis in numerous ways. First,

AGE and its interaction with RAGE reduces the levels of NO which is known to protect atherosclerosis through vasodilation, and inhibition of inflammatory mediators, platelet aggregation, and platelet activation.¹⁵⁰⁻¹⁵² Second, glycated lipoprotein B100 enhances the atherogenic activity of LDL.³⁹ Third, AGE-RAGE interaction decreases the reverse cholesterol transport.¹⁵³ Fourth, AGE and its interaction with RAGE produce ROS, NF- κ B, cytokines, adhesion molecules, MCP-1, GM-CSF, and growth factors which are involved in the development of atherosclerosis and has been described in detail in the previous section of this article. Besides these, it is to note that VCAM-1 induces activation of NADPH oxidase in the endothelium¹⁵⁴ which would increase the ROS levels and hence development of atherosclerosis.

AGE, RAGE, and sRAGE may play an important role in the development of atherosclerosis and hence CLLI. As described earlier in this article, plasma and skin²⁵⁻³⁴ levels of AGE and RAGE expression in tissue³³⁻³⁵ are elevated while the serum levels of sRAGE^{37,38} are reduced in patients with CLLI. In addition, AGE is present in the atherosclerotic plaque of diabetic patients.¹⁵⁵ sRAGE is antiatherogenic because it competes with RAGE for binding with AGE. Also, it interacts with AGE before RAGE can interact with AGE.¹⁵⁶ Thus, low sRAGE levels in patients with CLLI will bind with small amount of AGE and hence leaving more AGE available to interact with RAGE leading to development of atherosclerosis. Low levels of sRAGE is, therefore, atherogenic. It is known that atherosclerosis develops in diabetic patients in spite of high levels of sRAGE.^{157,158} One would have expected that high levels of sRAGE would have protected the development of atherosclerosis in diabetic patients but it did not do so. The reason could be that elevation of AGE levels is more than the elevation of sRAGE in diabetics. Hence, measurement of AGE and sRAGE in the same patient would be useful. Also, this will allow to assess the AGE-RAGE stress which is a ratio of AGE/sRAGE.²⁴ AGE-RAGE stress is a risk factor for disease and high AGE-RAGE stress indicates the presence and severity of the disease.

Therapeutic intervention based on the etiology of CLLI, should include reduction in the levels of AGE, prevention of AGE formation, degradation of AGE in vivo, suppression of RAGE expression, RAGE blockers, and elevation of sRAGE that have been described in detail earlier in this article. It has been reported that consumption of low AGE diet for 2 months reduces the serum levels of AGE in mice.¹⁵⁹ Reduction of AGE diet for a short duration reduced the serum levels of AGE in healthy and diabetic subjects.⁸² Stopping of cigarette smoking will also reduce the serum levels of AGE.⁸³ Exercise also reduces the levels of AGE in the serum.^{84,86,160,161} It has been reported that regular physical activity reduces AGE levels and diabetic complications.¹⁶² Unfortunately, no clinical trial has been conducted to examine the effectiveness of agents that reduce the formation of AGE or reduce the levels of AGE in reduction of atherosclerotic changes in patients with CLLI. Although some of the agents (benfotiamine,¹⁶³ vitamin E^{138,164}) have been shown to reduce the atherosclerotic changes in animal studies and humans. Combined use of vitamin E and vitamin C has been shown to slow down the progression of atherosclerosis in hypercholesterolemic subjects.¹⁶⁵ Combination of vitamin E

and vitamin C will be effective in reducing atherosclerosis in two ways: by scavenging ROS and by reducing the formation of AGE.

Enzymatic (GLO1 and GLO2) degradation and AGE receptor-mediated (AGER1 and AGER2) degradation of AGE would reduce the levels of AGE in the body. However, no such pharmaceuticals have been developed for use in patients. Attempt should be made by pharmaceutical companies to develop these degraders for use in humans.

There are drugs used in diabetic and hypertensive patients which suppress the expression of RAGE. Some of the patients with CLLI may already have been using these drugs and are getting the benefits. Azeliragon (a RAGE blocker) has been developed¹¹⁴ and has been shown to be effective in improving cognitive function in patients with Alzheimer's disease.¹¹³ May be this drug would work for patients with CLLI. A search for new RAGE blocker should be intensified.

The levels of sRAGE in blood should be elevated by increasing expression of sRAGE and by exogenous administration in patients with CLLI. The drugs (statins, angiotensin-converting enzyme inhibitors, antidiabetic drug, and rosiglitazone) elevate the sRAGE expression. Again some of the patients with CLLI may be using these drugs for other associated conditions, and taking advantage of this. Exogenous administration of sRAGE has been shown to be effective in preventing the development of atherosclerosis in animal studies.^{7,8,131,132} Exogenous administration of sRAGE should be tried in animal model of CLLI to see if it is effective in prevention, regression, and slowing of atherosclerosis. Also, recombinant sRAGE should be developed for use in humans, and tried in patients with CLLI.

These treatment modalities may not be fully effective in patients with CLLI because some other factors besides AGE-RAGE axis may also be involved in the pathogenesis of CLLI.

Conclusion

CLLI is due to atherosclerosis in the lower limb arteries. Elevated levels of AGE in serum/plasma, increased expression of RAGE in arteries, and reduced serum levels of sRAGE are involved in the development of atherosclerosis through oxidation of LDL, reduction in NO, activation of NF- κ B, and increases in the levels of ROS, cell adhesion molecules, cytokines, MCP-1, GM-CSF, and growth factors. The treatment modalities (prevention, regression, and slowing of progression of atherosclerosis) of CLLI should include lowering of AGE consumption, prevention of AGE formation, increase in degradation of AGE in vivo, suppression of RAGE, RAGE blocker, upregulation of sRAGE expression, and exogenous administration of sRAGE.

Disclosure

None.

Conflict of Interest

None.

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