Anti-atherosclerotic effect of aged garlic extract: Mode of action and therapeutic benefits (Review)

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Abstract. Atherosclerosis, a chronic inflammatory disease characterized by plaque buildup within the arteries that obstructs blood flow and significantly increases the morbidity and mortality rates associated with cardiovascular diseases caused by impaired blood flow due to vascular stenosis or occlusion, such as angina and myocardial infarction. The development of atherosclerosis involves a complex interplay of endothelial dysfunction, accumulation of oxidized low-density lipoprotein and macrophage-driven inflammation. The risk factors for atherosclerosis include chronic inflammation, hyperlipidemia and hypertension. Effective management of these risk factors can prevent and delay the onset and progression of atherosclerosis. Garlic and its processed preparations have previously been utilized to mitigate cardiovascular risk factors and continue to be used in traditional medicine in several countries. Among these preparations, aged garlic extract (AGE) has been shown to improve atherosclerosis in clinical trials and animal studies. AGE contains various compounds with potential anti-atherosclerotic properties, such as *S*-1-propenylcysteine, *S*-allylcysteine and other sulfur-containing constituents, which may help prevent the development and progression of atherosclerosis. The present manuscript reviewed and discussed the anti-atherogenic effect of AGE and its constituents by highlighting their mode of action and potential benefits for prevention and therapy in the management of atherosclerosis.

Contents

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1. Introduction

Cardiovascular diseases (CVDs) including coronary heart disease, cerebrovascular disease and rheumatic heart disease are the leading cause of death worldwide, claiming ~17.9 million lives annually. The major cause of CVDs is atherosclerosis, an inflammatory disease that occurs in vessel walls (1,2). The development of arteriosclerosis is associated with various factors, including increased shear stress due to hypertension (3,4), inflammation induced by damage-associated molecular patterns (DAMPs) from dead cells (5-7) and augmentation of oxidation and glycation products resulting from hyperlipidemia including hypercholesterolemia (8,9) and hyperglycemia (10,11). These factors contribute to the damage of vascular endothelial cells, which subsequently leads to the accumulation of low-density lipoprotein (LDL) cholesterol and migration of circulating monocytes into the blood vessel wall (12,13). The infiltrated monocytes differentiate into macrophages, which phagocytose oxidized LDL (oxLDL) and become foam cells, leading to the formation of plaque with a lipid core (14,15). Subsequently, the progression of fibrosis and calcification of the plaque make the plaque unstable and prone to rupture. Plaque rupture and subsequent thrombus formation can trigger cerebral or myocardial infarction (16). Thus, proper reduction of these risk factors can help prevent and/or slow both the onset and progression of atherosclerosis.

Garlic has been previously reported to be effective in inhibiting the pathogenesis of CVDs and to help prevent chronic diseases such as diabetes mellitus, cranial nerve disease and cancer (17). Aged garlic extract (AGE), prepared by aging crushed raw garlic in water-soluble ethanol for at least 10 months, contains a variety of compounds produced through aging processes. These constituents include *S*-alk(en)

ylcysteine compounds such as *S*-allylcysteine (SAC), *S*-1-propenylcysteine (S1PC™; Wakunaga Holdings Co., Ltd.), *S*-allylmercaptocysteine (SAMC), *S*-propylcysteine (SPC) and *S*-ethylcysteine (SEC); diallyl polysulfide compounds such as diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl trisulfide (DATS); Maillard reaction-related compounds such as fructosyl-L-arginine (Fru-Arg); phenolic compounds such as dehydrodiconiferyl alcohol (DDC) and dihydrodehydrodiconiferyl alcohol (DDDC); and amino acids such as L-arginine, L-cysteine and L-methionine (Fig. 1) (18,19). However, AGE contains fewer irritating compounds, such as allicin, when compared with raw garlic and causes little damage to the gastric mucosa (20,21). Efendy *et al* (22) first reported in 1997 that AGE inhibits the development of experimental atherosclerosis in rabbits. Since then, the anti-atherosclerotic effects of AGE have been demonstrated in various clinical (23-36) and animal studies (19,22,37-40). For example, clinical trials have shown that AGE can reduce lipid-rich low attenuation plaque by ~30% (24,26) and inhibit the progression of vascular calcification by ~65% (27,29,30).

This review aimed to highlight the beneficial effects and underlying mechanisms of action of AGE and its active constituents in mitigating risk factors associated with the onset of atherosclerosis and modulating key processes in its progression. Additionally, the clinical relevance, availability and potential applications of AGE in the prevention and treatment of atherosclerosis was explored.

2. Effect of AGE and its constituents on risk factors for atherosclerosis

Atherosclerosis is a complex multifactorial disease triggered by several risk factors, such as chronic inflammation, hypercholesteremia and hypertension. The prevention and treatment of atherosclerosis requires the control of risk factors. In the following section, the effect of AGE and its key constituents on these risk factors will be reviewed (Fig. 2).

Chronic inflammation. Chronic inflammation associated with obesity and aging is characterized by persistent and prolonged low-grade inflammation, often triggered by DAMPs released from dead cells (41,42). This type of inflammation serves a crucial role in the initiation and progression of atherosclerosis. Furthermore, autoimmune diseases such as systemic lupus erythematosus, antiphospholipid syndrome and rheumatoid arthritis, which also induce chronic inflammation, are associated with an increased risk for atherosclerosis. Patients with these autoimmune diseases face a significantly increased risk of developing new atherosclerotic plaques, 3.3-50.0 times higher compared with healthy individuals (43,44). Notably, the production of DAMPs, which mediate chronic inflammation, is influenced by various factors, such as smoking, obesity and hyperlipidemia (6). The key DAMPs involved in this process include the chromatin protein high mobility group box 1, S100 calcium-binding protein A (S100A) 8, S100A9, S100A12 and oxLDL (5,6). These molecules further perpetuate the inflammatory response, contributing to the progression of atherosclerosis. In addition, these DAMPs serve a critical role in inducing the release of pro-inflammatory cytokines, such as IL-6 and TNF-α. The recognition and binding of DAMPs by pattern recognition receptors, particularly Toll-like receptor 4 (TLR4) and receptor for advanced glycation end products, are central to this inflammatory response. This pathway underscores the complex interplay between chronic inflammation and the pathophysiological processes driving atherosclerosis, DAMPs being key mediators in both the initiation and progression of the disease (7,45,46).

Previous studies have demonstrated that AGE induced a 20% decrease in IL-6 production in patients with coronary artery calcium scores <5 and in healthy adults with obesity (47,48). In addition, *in vitro* studies have reported that several AGE-constituents, S1PC, DADS and DATS inhibit IL-6 production induced by lipopolysaccharide (LPS), a TLR4 agonist (49-52). Among these components, DADS and DATS suppress TLR4 signaling by inhibiting NF-κB, whereas S1PC acts through a distinct mechanism. Specifically, S1PC induces the degradation of the adapter protein myeloid differentiation primary response 88 by activating autophagy (51,52). Thus, S1PC has been reported to exhibit anti-inflammatory effects through a novel mechanism involving the suppression of TLR4 signaling by activating autophagy. However, since the mechanism of autophagy activation by S1PC is not yet fully understood, the anti-inflammatory effects mediated by this mechanism require further investigation. In addition, several AGE constituents have been reported to inhibit autoimmune diseases. For example, DADS prevents cartilage destruction, ameliorates arthritis and reduces inflammation by decreasing the expression of pro-inflammatory cytokines in arthritis rat models (53). Furthermore, L-arginine improves arthritis and mitigates inflammatory bone loss by reducing the number of osteoclasts (54).

These findings suggest that AGE may suppress chronic inflammation, a key risk factor for atherosclerosis by suppressing TLR signaling, which is a primary trigger of inflammation, potentially acting prophylactically to inhibit the progression of atherosclerosis.

Hypercholesterolemia. Hypercholesterolemia contributes to the development of atherosclerosis. The risk for atherosclerosis increases by 2-3% for each 1% rise in the serum cholesterol level (55,56). Conversely, lowering serum cholesterol by 10% can reduce the risk for atherosclerosis by 50% in 40 year old men and by 25% in 60 year old men over a 5 year period according to the results of an epidemiological survey (55,56). Hypercholesterolemia enhances the production of reactive oxygen species (ROS), which promotes the secretion of several pro-inflammatory cytokines, including IL-1, IL-2, IL-6, IL-8, TNF-α and IFN-γ, by activating NF-κB (55,57,58). Supplementation with AGE alone or in combination with B vitamins has been shown to decrease total cholesterol (TC) level by ~7% in clinical studies (36,59-61) and ~15% in animal studies (40,59). AGE has also been shown to inhibit cholesterol synthesis in rat hepatocytes, thus it is suggested that the cooperative action of several components of AGE, such as SAC, SPC, SEC, γ-glutamyl SAC and γ-glutamyl SPC, may contribute to its effect (59). These studies suggest that AGE decreases TC, which may contribute to the prevention of atherosclerosis.

Hypertension. Hypertension has been shown to significantly elevate the risk for developing atherosclerosis in clinical

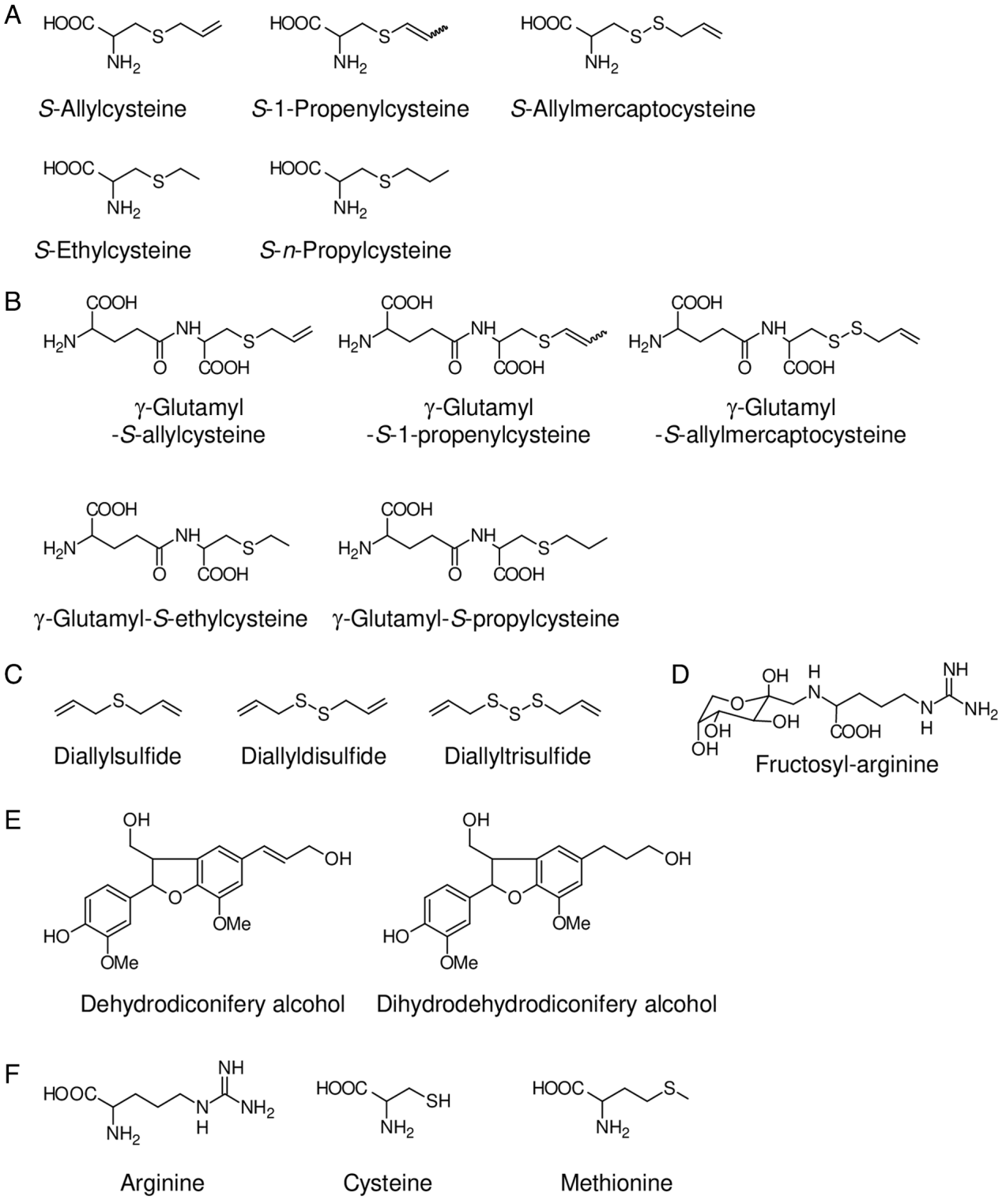

Figure 1. Chemical structure of major constituents in aged garlic extract. (A) *S*-Alk(en)ylcysteine, (B) ɤ-glutamylpeptide derivatives, (C) allylpolysulfides, (D) maillard reaction-relating compounds, (E) phenolic compounds and (F) amino acids.

trials (62). According to epidemiological research, arterial hypertension was identified as the most crucial cardiovascular risk factor, contributing to 48% of all strokes and 18% of all coronary events (62). A randomized trial with 3,845 participants, averaging 83 years in age, demonstrated that reducing blood pressure from 161/84 to 144/78 mmHg decreased the risk for cerebral circulatory disorders by 30% and cardiovascular events by 23% (62,63). Additionally, in patients with vascular disease or diabetes mellitus plus an additional cardiovascular risk factor, treatment with ramipril, an angiotensin-converting enzyme inhibitor, resulted in a 22% reduction in the composite endpoint of cardiovascular death, myocardial infarction and stroke (64). Therefore, antihypertensive therapy is important for the prevention or improvement of atherosclerosis. AGE has been reported to improve not only atherosclerosis but also hypertension in several clinical trials. The AGE-treated group

demonstrated a decreased mean systolic blood pressure (SBP) by ~10 mm Hg after 12 weeks of administration (65-67). In addition, it has been reported that active components of AGE, including S1PC and SAC, reduce blood pressure. The repeated administration of S1PC for 10 weeks significantly decreases SBP of spontaneously hypertensive rats (68). Furthermore, it was reported that the single administration of S1PC reduces SBP after 3 h via the central histamine H3 receptor by altering histidine metabolism (69,70). Moreover, administration of SAC has been shown to decrease SBP in both ovariectomized and five-sixths of a group of nephrectomized rats (71,72). These results suggested that AGE may help prevent atherosclerosis by mitigating hypertension, which is a risk factor for the development of atherosclerosis.

Since AGE contains multiple bioactive components that act on various targets through different mechanisms, they have the potential to simultaneously improve major risk factors for atherosclerosis, such as chronic inflammation, dyslipidemia and hypertension. By acting on these factors, AGE may help prevent the progression of atherosclerosis.

3. Effect of AGE and its components on vascular endothelial function

Vascular endothelial cells, located on the innermost layer of blood and lymphatic vessels, serve crucial roles in delivering oxygen and nutrients, regulating blood flow, modulating immune cell trafficking and maintaining tissue homeostasis (73). C-reactive protein (CRP), induced by inflammation, can cause endothelial dysfunction by directly damaging endothelial cells and reducing the number and function of endothelial progenitor cells (43,44,74). Endothelial injury triggers ROS production and vascular inflammation, increasing the expression levels of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) on the cell surface and the secretion of C-C motif chemokine ligand 2 (CCL2) (75). This disrupts tight junctions, which normally restrict the entry of circulating substances and immune cells from the bloodstream into the vessel wall, allowing monocytes to infiltrate the vessel wall (76). Non-inflammatory $Ly6C^{low}$ monocytes typically patrol the vasculature to phagocytose and scavenge debris and maintain endothelial integrity. By contrast, the number of inflammatory $Ly6C^{hi}$ monocytes increases during chronic inflammation and hypercholesterolemia, preferentially adhere to activated endothelium, infiltrate the vessel wall and differentiate into lesional macrophages (77-80).

This infiltration of $Ly6C^{hi}$ monocytes corresponds to the early stages of atherosclerosis development, with $Ly6C^{hi}$ monocytes eventually transforming into foam cells that accumulate in blood vessels (Fig. 2A) (76-79). The following mechanisms of actions discussed relate to the effects of AGE and its components in protecting vascular endothelial function.

Antioxidative effect on vascular endothelial cells. AGE, SAC and Fru-Arg have been reported to inhibit the production of H_2O_2 and lipid peroxides induced by oxLDL through ROS. These compounds promote the nuclear accumulation of nuclear factor erythroid 2-related factor 2 (Nrf2), which is a transcription factor activated in response to oxidation, and increase the gene and protein expression level of the antioxidant enzymes heme oxygenase 1 (HO-1) and glutamate-cysteine ligase modifier subunit (GCLM) in human umbilical vein endothelial cells (HUVECs), thereby improving endothelial dysfunction. This change is accompanied by an increase in the intracellular level of the antioxidant glutathione, suggesting that AGE and its constituents exert antioxidant activity to prevent oxLDL-induced oxidation and cellular damage (81-86). It has also been shown that S1PC enhances activation of the Nrf2 pathway in the presence of a nitric oxide (NO) donor by promoting the degradation of broad complex, tramtrack and bric-a-brac domain and cap'n'collar homology 1, a transcriptional repressor of Nrf2 (87,88). Additionally, SAC has been shown to activate endothelial nitric oxide synthase (eNOS) in endothelial cells and promote NO production (89,90). These results suggest that AGE and its constituents may act together to increase the cellular antioxidant capacity through enhancement of the Nrf2 pathway and ameliorate the vascular endothelial cell dysfunction and exacerbated inflammation caused by oxidation (Fig. 3A).

Monocyte adhesion. DDC and DDDC, identified as antioxidants in AGE, have been shown to suppress VCAM-1 expression induced by LPS or advanced glycation end products in HUVECs by inhibiting the JNK/c-Jun pathway, but not the NF-κB pathway, thereby preventing the adhesion of THP-1 monocytes to the surface of HUVECs (91). Similarly, DAS, DADS and DATS, which are minor constituents of AGE (18), suppress oxLDL-induced VCAM-1 and E-selectin expression levels on the cell surface, reducing the adhesion of the human promyelocytic leukemia cell line HL-60 to HUVECs (92). Notably, the inhibitory potency of these sulfur-containing compounds increases with the number of sulfur atoms, in the order of DATS > DADS > DAS (92). Their mechanisms involve activation of the PI3K/protein kinase (PK) B signaling pathway to suppress E-selectin expression and dephosphorylation of PKA and cAMP response element binding protein to reduce VCAM-1 expression levels, each of which is mediated through the PKB/PI3K signaling pathway (92). Additionally, AGE and S1PC inhibit the secretion of CCL2, a chemokine that attracts monocytes (51,93). In addition, L-arginine, a major amino acid in AGE, suppresses IL-1β-induced VCAM-1 and ICAM-1 expression, inhibiting the adhesion of human peripheral blood-derived monocytes to HUVECs (94). L-arginine also exhibits anti-atherosclerotic effects, such as reducing lipid deposition in the aorta, improving flow-dependent vasodilation and preventing monocyte adhesion to the vascular surface in a hypercholesterolemic rabbit atherosclerosis model (95,96).

These findings suggest that multiple constituents in AGE suppress the adhesion of monocytes to vascular endothelial cells by not only reducing the expression of adhesion molecules on endothelial cells but also inhibiting the secretion of chemokines, such as CCL2, that attract monocytes (Fig. 3A).

Endothelial barrier function. Vascular endothelial cells adhere to each other through adherens junctions including vascular endothelial (VE)-cadherin, and tight junctions including claudin, occludin and zonula occludens-1 (ZO-1). These junctions restrict the entry of circulating substances and immune cells from the blood into the vessel wall (13). Disruption of this barrier function by inflammation and oxidation leads to the

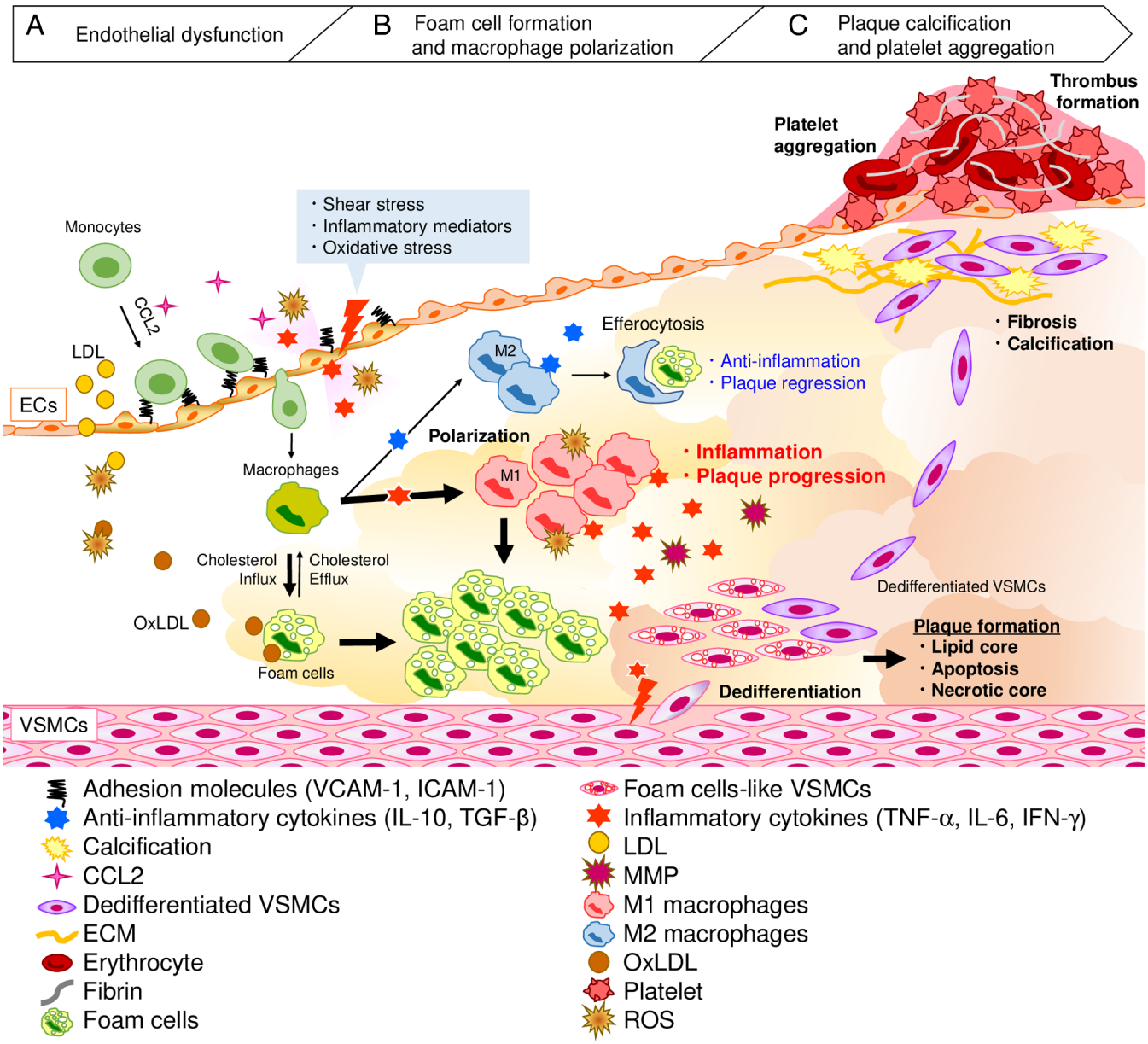

Figure 2. Process of plaque formation. (A) Endothelial dysfunction. Damage to vascular endothelial cells by inflammatory mediators and ROS reduces endothelial barrier function and induces infiltration of circulating monocytes and lipids into the vascular intima. (B) Foam cell formation and macrophage polarization. Infiltrating monocytes differentiate into macrophages, which take up oxLDL via scavenger receptors, including CD36, to form foam cells. Macrophages polarize into inflammatory M1 macrophages and anti-inflammatory M2 macrophages depending on the plaque microenvironment. M1 macrophages release inflammatory factors to exacerbate inflammation, promoting plaque formation, while M2 macrophages release anti-inflammatory cytokines and efferocytose foam cells and apoptotic cells, contributing to plaque regression. (C) Plaque calcification and platelet aggregation. Dedifferentiated VSMCs, which have been dedifferentiated by exacerbated inflammation, migrate and proliferate in the intima, inducing uptake of oxLDL and fibrosis and calcification on the plaque surface. As the disease progresses and the plaque becomes unstable, platelets adhere and aggregate at the site of endothelial cell loss, forming a thrombus that can occlude blood vessels and induce cardiovascular disease. CCL2, C-C motif chemokine ligand 2; ECM, extracellular matrix; ECs, endothelial cells; ICAM-1, intercellular adhesion molecule-1; LDL, low-density lipoprotein; MMP, matrix metalloproteinase; oxLDL, oxidized LDL; ROS, reactive oxygen species; VCAM-1, vascular cell adhesion molecule-1; VSMCs, vascular smooth muscle cells.

entry of lipids and circulating immune cells into the intima, increasing lipid deposition and vascular inflammation in the aorta (12,13). Kunimura *et al* (97) reported that AGE and S1PC, but not SAC and SAMC, inhibited cell permeability by suppressing TNF-α-induced downregulation of VE-cadherin, claudin-5 and ZO-1 through the suppression of the Rho guanine nucleotide exchange factor-H1/RhoA/Rac pathway in HUVECs. These results suggest that AGE prevents the disruption of tight junctions caused by inflammation and maintains the integrity of intercellular adhesion (Fig. 3A).

In summary, AGE and its components maintain vascular endothelial barrier function and prevent monocyte adhesion and infiltration, which may consequently inhibit atherosclerotic plaque development (Fig. 3A).

4. Effect of AGE and its constituents on foam cell formation

LDL in blood enters the vascular intima through the gaps between endothelial cells, where it is oxidized by ROS derived from vascular endothelial cells and macrophages, and is

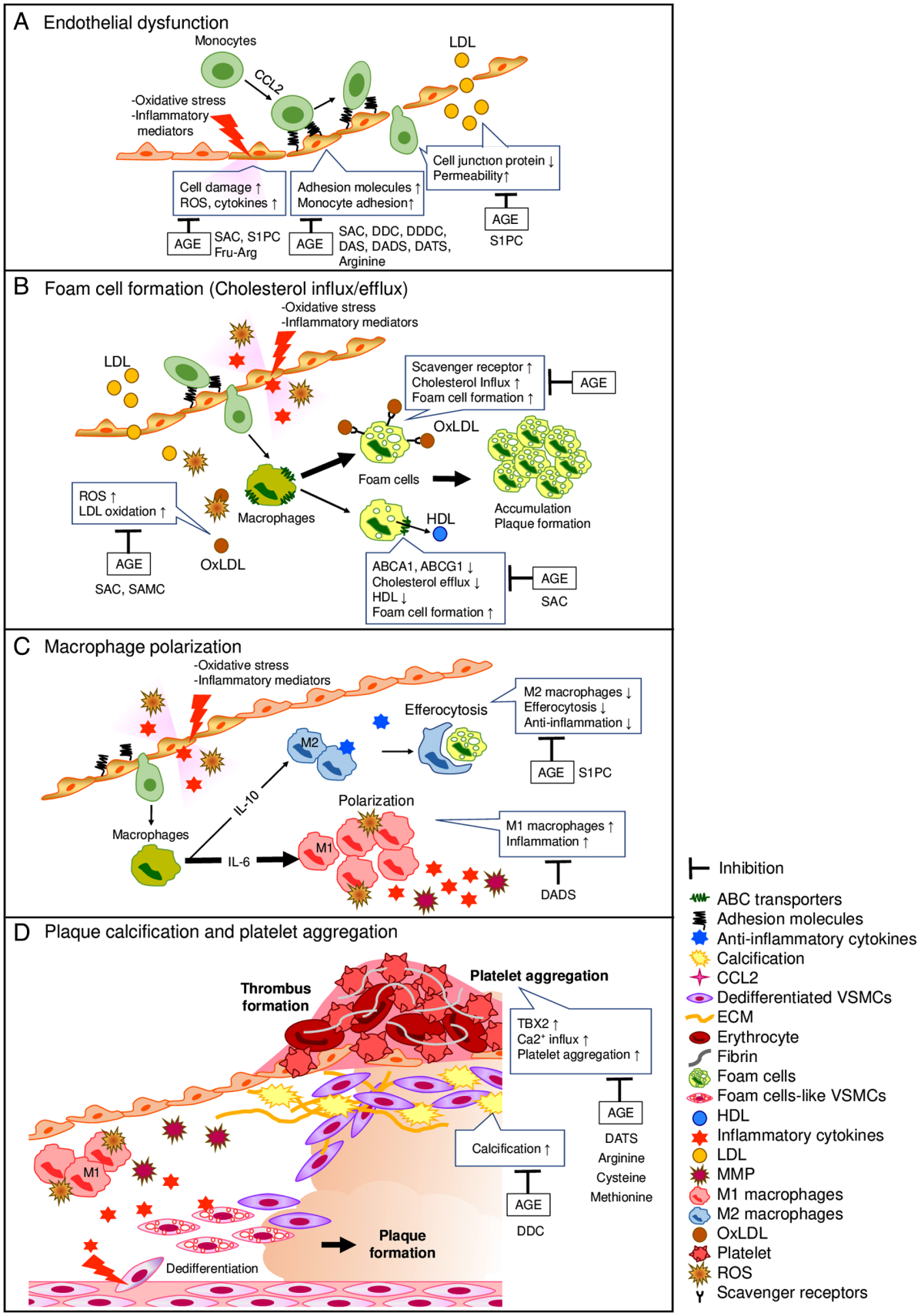

Figure 3. Effect of AGE and its constituents in the processes of plaque formation. The inhibitory effects of AGE and their constituents on the plaque formation processes. (A) Endothelial dysfunction, (B) foam cell formation (macrophage cholesterol influx/efflux), (C) macrophage polarization and (D) plaque calcification and platelet aggregation. ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; AGE, aged garlic extract; CCL2, C-C motif chemokine ligand 2; DADS, diallyl disulfide; DAS, diallyl sulfide; DATS, diallyl trisulfide; DDC, dehydrodiconiferyl alcohol; DDDC, dihydrodehydrodiconiferyl alcohol; ECM, extracellular matrix; Fru-Arg, fructosyl-L-arginine; HDL, high-density lipoprotein; IL, interleukin; LDL, low-density lipoprotein; MMP, matrix metalloproteinase; oxLDL, oxidized LDL; ROS, reactive oxygen species; SAC, *S*-allylcysteine; SAMC, *S*-allylmercaptocysteine; S1PC, *S*-1-propenylcysteine; TBX_2, thromboxane B2; VSMCs, vascular smooth muscle cells.

subsequently deposited in the vascular intima as oxLDL (2). Macrophages take up oxLDL via scavenger receptors (SRs) such as SR-AI, CD36 or lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1), and the excessive uptake of oxLDL transforms macrophages into foam cells, resulting in the development of an atherosclerotic plaque (Fig. 2B) (14-16). Atherosclerotic lesions were reduced in an atherosclerosis model of mice lacking theses SRs (98-100). Thus, the suppressed expression of SRs in macrophages may contribute to the inhibition of foam cell formation and plaque development. It has been reported that AGE suppresses peroxisome proliferator-activated receptor γ-mediated CD36 expression and inhibits the intracellular uptake of oxLDL in macrophages derived from the human monocyte THP-1 cell line (101-103). On the other hands, macrophages also efflux free cholesterol (FC) converted from oxLDL via ABC transporters, such as ABCA1 and ABCG1, which are necessary for high-density lipoprotein (HDL) synthesis. This FC is subsequently incorporated into HDL and transported back to the liver (15,104). In clinical trials and animal experiments, AGE increased plasma HDL-cholesterol concentration, suggesting that it may improve cholesterol metabolism (36,40,59-61). In addition, SAC increased ABCA1 gene and protein expression levels in THP-1-derived macrophages, which may improve cholesterol metabolism (105). It has been reported that depletion of macrophage-specific ABCA1 and ABCG1 exacerbates plaque formation (106-108), whereas overexpression of ABCA1 is protective against atherosclerosis (109).

Thus, AGE may reduce cholesterol accumulation in macrophages by suppressing the expression of SRs and increasing the expression of ABC transporters. These results also suggest that AGE inhibits the progression of atherosclerotic lesions by reducing foam cell formation (Fig. 3B).

5. Effect of AGE and its constituents on macrophage polarization

Macrophages polarize into two major phenotypes: Inflammatory M1 macrophages and anti-inflammatory M2 macrophages, depending on the arterial plaque microenvironment (110). In plaque lesions, M2 macrophages are more dominant compared with M1 macrophages from the early stages of plaque formation until plaque stabilization. They contribute to plaque stabilization through the efflux of cholesterol, production of anti-inflammatory cytokines, phagocytosis of apoptotic cells and collagen production induced by TGF-β, thereby regulating plaque progression (110). However, as plaque formation progresses and a lipid and necrotic core is formed, M1 macrophages become dominant. This shift leads to the production of inflammatory cytokines, increased lipid accumulation due to decreased cholesterol efflux and thinning of the cap caused by the production of matrix metalloproteinases, resulting in an unstable plaque and an increased risk of rupture (Fig. 2B) (111-113). Crocin, an active ingredient of *Crocus sativus L*., and pomegranate juice increased the number of M2 macrophages and inhibited the progression of aortic plaque formation in atherosclerotic mice (114,115). Therefore, it is important to maintain the predominance of M2 macrophages over M1 macrophages to inhibit plaque progression or to stabilize plaques (110,116).

AGE has been reported to decrease the expression levels of M1 macrophage markers and increase the expression levels of M2 macrophage markers in aortic and splenic lymphocytes. The active component responsible for this effect of AGE is S1PC, which has been shown *in vitro* to prolong IL-10-mediated STAT3 activation, thereby promoting polarization into M2c macrophages with a high IL-10 production capacity (38). Additionally, DADS, another active component of AGE, reduces the number of M1 macrophages by suppressing LPS-induced NF-κB activation through the Nrf2 pathway, thereby inhibiting polarization into M1 macrophages and reducing inflammation (117).

However, since macrophage polarization is influenced by the microenvironment, it is crucial to investigate whether AGE or its components can induce M2 macrophage polarization within the context of a chronic inflammatory environment, such as that found in atherosclerotic plaques. Notably, clinical trials have demonstrated that drugs such as pioglitazone and thiazolidinediones, which promote polarization towards M2 macrophages, significantly suppressed atherosclerosis in patients with type 2 diabetes (116,118-120). Consequently, therapeutic strategies targeting M2 macrophage polarization have emerged as promising avenues for atherosclerosis treatment (116). These clinical observations suggest that AGE and its components might contribute to atheroprotection by promoting a shift towards the M2 macrophage phenotype (Fig. 3C). However, the polarization of macrophages within atherosclerotic plaques is a complex process involving various subpopulations. Further research is needed to pinpoint the specific macrophage subsets affected by AGE and to elucidate the underlying molecular mechanisms that drive these changes.

6. Effect of AGE and its constituents on vascular calcification and platelet aggregation

Vascular calcification. Vascular smooth muscle cells (VSMCs) are typically present in the vascular media and are involved in vascular contraction and relaxation. However, they dedifferentiate and migrate into the vascular intima in response to cytokines, growth factors released from damaged vascular endothelial cells and activated macrophages (121,122). Migrated VSMCs proliferate in the vascular intima and change to various phenotypes, such as foam cell-like and osteoblast-like cells, and are involved in the development of plaque formation (121,122). In calcified plaque lesions, VSMCs upregulate the expression of osteogenesis-related factors including alkaline phosphatase (ALP), runt-related transcription factor 2, osteopontin and bone morphogenetic protein 2, suggesting that the proliferation of osteoblast-like VSMCs contributes to plaque calcification (Fig. 2C) (122).

Several clinical trials have reported that AGE administered for 1 year improved the coronary artery calcification scores of patients with coronary artery disease (27,29,30,123), suggesting that AGE may regulate the activation of osteoblast-like VSMCs. In addition, DDC has been shown to significantly inhibit ALP activity induced by culturing human coronary artery smooth muscle cells with dexamethasone and the culture supernatant of THP-1 derived macrophages (124). These findings suggest that AGE and DDC may inhibit plaque calcification by suppressing the osteogenic differentiation of VSMCs (Fig. 3D) (27,29,30,123,124).

Platelet aggregation. Plaque rupture and subsequent thrombus formation trigger arterial stenosis and disrupt blood flow in the late stage of atherosclerosis. Platelets bind to von Willebrand factor (VWF) present in the exposed subendothelial collagen layer due to plaque rupture via the glycoprotein (GP) complexes. Platelets adhering to subendothelial tissue release ADP, thrombin and thromboxane A2, promoting platelet activation. Activated platelets bind to VWF and fibrinogen via GP complex (GPIIb/IIIa) to form platelet aggregates, which become thrombi (125,126). The thrombus is stabilized by fibrin, which is produced from fibrinogen, and promotes the blood clotting reaction on the platelet membrane (125-127). This thrombus can cause stenosis, occlusion or dissection of blood vessels (Fig. 2C).

Steiner and Li (128) reported that in the blood of patients with moderate hypercholesterolemia taking AGE for 6 weeks, collagen-, epinephrine- and ADP-induced platelet aggregation was reduced, and platelet adhesion to collagen, VWF and fibrinogen was inhibited. Additionally, ADP-induced platelet aggregation was suppressed in the blood of normolipidemic subjects who took AGE for 13 weeks (129). Although AGE inhibits platelet aggregation, no serious adverse events have been reported when AGE is used in combination with the anticoagulant warfarin, indicating it may be relatively safe to use (130). Furthermore, serum from apolipoprotein E-knockout mice, whose atherosclerosis improved after 12 weeks of AGE feeding, showed a significant decrease in the concentration of thromboxane B2, a marker of platelet activation, suggesting that AGE may inhibit platelet aggregation by suppressing platelet activation (39). Platelets are activated through MAPK kinase by several agonists, such as ADP and collagen (131-133). It has been reported that platelets from rats treated with AGE for 2 weeks exhibit inhibited collagen-induced platelet aggregation and suppressed the phosphorylation of ERK, p38 and JNK (134). In addition, studies on human platelets have suggested that AGE may inhibit platelet activation by reducing ADP-induced Ca^{2+} influx into the cell, thereby inhibiting GPIIb/IIIa activation (135-137). It has been reported that L-arginine, L-cysteine and L-methionine, which are components of AGE, inhibit ADP-induced platelet aggregation, while DATS inhibits collagen- and thrombin-induced platelet aggregation (19,138). Therefore, it is possible that AGE may inhibit atherothrombosis by suppressing platelet aggregation through the collaborative action of its multiple pharmacologically active components (Fig. 3D).

These findings suggest that AGE may prevent plaque rupture by inhibiting calcification of advanced atherosclerotic lesions. Additionally, if plaques do rupture, AGE may inhibit platelet aggregation, thereby reducing the risk of subsequent myocardial infarction and angina pectoris (Fig. 3D).

7. Future directions and limitations of current assessments

Establishing the causal relationship between AGE intake and its diverse pharmacological effects in clinical trials has inherent limitations due to the complexity of its mechanisms of action and various confounding factors. Furthermore, the underlying mechanisms driving AGE's broad biological activities remain largely elusive, in part due to the presence of numerous bioactive compounds. Developing robust targeting and screening systems is essential for identifying these active constituents and their specific roles. A comprehensive understanding of AGE's multifaceted biological effects is therefore imperative, and OMICS-based approaches, including proteomics and miRNA analysis using clinical, animal and cell samples, could facilitate this process by identifying key molecular targets and regulatory pathways (139-144).

In clinical studies conducted to date, the effect of AGE has been evaluated for up to 1 year; however, its effects on the onset and progression of atherosclerosis are expected to become clearer with longer-term follow-up studies. Therefore, large, randomized, double-blind clinical trials with long-term treatment and follow-up periods are needed to assess the effects of AGE on the development and progression of atherosclerosis, as well as their impact on the clinical outcomes of patients with atherosclerosis. Additionally, the dosage of AGE in clinical trials for atherosclerosis treatment varies between 1,000-2,400 mg/day, depending on the trial (27,29,30,123). Determining the optimal dosage for each target disease through dose-response testing and similar assessments remains a critical challenge.

To advance AGE research, it is essential to comprehensively characterize its bioactive components, their kinetics and their precise mechanisms of action. Future investigations should also focus on identifying key active constituents, elucidating their molecular targets and exploring AGE's potential synergistic effects with existing drugs, its applicability to clinical trials and its relevance beyond atherosclerosis.

8. Conclusion

The multifactorial mechanism involving endothelial dysfunction, oxLDL accumulation, macrophage-induced inflammation and other risk factors in the development of atherosclerosis makes it difficult to prevent and treat the disease with a single target or mechanism. In this context, AGE has potential therapeutic and preventive applications, as it contains multiple active components and causes few side effects. AGE and its components exhibit diverse mechanisms of action that affect various aspects of disease progression, such as reducing risk factors like chronic inflammation, hyperlipidemia and hypertension, suppressing endothelial dysfunction, reducing oxLDL formation and increasing in HDL levels, reducing foam cell formation and promoting M2 macrophage polarization and suppressing platelet aggregation.

These effects of AGE can be attributed to its pharmacologically active sulfur-containing components, which have demonstrated inhibitory actions on various stages of atherosclerosis progression. By acting on multiple pathways simultaneously, AGE exhibits a unique multi-target approach to atherosclerosis prevention and treatment, with its constituents acting synergistically (Fig. 3). Notably, AGE has also been shown to delay coronary artery calcification in patients undergoing statin therapy without affecting side effects, suggesting its potential to complement the effects of existing atherosclerosis drugs.

Despite promising preclinical and clinical evidence, the intricate mechanisms underlying the diverse biological activities of AGE remain largely elusive. Contributing to this complexity is the presence of a wide array of bioactive compounds within

AGE. Developing robust targeting and screening systems is crucial for identifying these active constituents. To this end, a comprehensive understanding of the multifaceted biological effects of AGE is imperative. Future research should utilize chemical proteomics and network pharmacology approaches to identify active constituents, target molecules and mechanisms of action, thereby elucidating the effects of AGE. A comprehensive characterization of AGE is essential, and these findings should be leveraged to explore its potential synergistic effects with existing drugs, its applicability to clinical trials and its relevance beyond atherosclerosis.

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Author's contributions

SM and JIS conceived this review. SM, MT and JIS analyzed the relevant literature. SM and JIS wrote the manuscript. SM constructed the figures. MT and JIS critically revised the manuscript. All authors read and approved the final version of the manuscript. Data authentication is not available.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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