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REVIEWS: CURRENT TOPICS

Mechanisms of anti-atherosclerotic functions of soy-based diets☆

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Abstract

Soy-based diets have been reported to protect against the development of atherosclerosis. However, the underlying mechanism(s) for this protection remains unknown. Although atherosclerosis was traditionally considered a disease associated with impaired lipid metabolism, in recent years the inflammatory components of atherosclerosis have been explored. Recent studies have convincingly delineated that uncontrolled chronic inflammation is the principal contributing factor for the initiation and progression of atherosclerosis. Interaction between activated monocytes and vascular endothelial cells is an early event in atherogenesis. The adhesion of leukocytes, including monocytes, to the inflamed-vascular endothelium and their transmigration into intima initiate the inflammatory processes. Following transmigration, monocytes in the intima are transformed to macrophages, which take up oxidized-LDL (oxLDL) to generate lipid-laden macrophages, also known as foam cells. Hence, in this review article the inflammatory processes associated with atherosclerosis and possible anti-inflammatory functions of soy-based diets contributing to the prevention of atherosclerosis are presented.

Keywords: Soy; Atherosclerosis; Isoflavones; Inflammation; Cell adhesion

1. Introduction

Cardiovascular disease (CVD) continues to be the leading cause of death in the US and other Western countries. Atherosclerosis, thickening of arterial intima, is one of the most common causes of CVD. Recent studies have suggested that maternal hypercholesterolemia during pregnancy is associated with a marked increase in aortic fatty streak formation in human fetuses and a more rapid progression of atherosclerosis during normocholesterolemic childhood [1–4]. Furthermore, with the increasing incidence of type II diabetes and childhood obesity, atherosclerosis has become a major health problem in the US and in many developing nations [5,6]. Hence, the prevention of atherosclerosis by dietary intervention would reduce the early onset and incidence of CVD.

1.1. Soy and cardiovascular disease

Epidemiological studies have shown a lower incidence of CVD in Asia than in Western countries [7,8] that is associated with a reduced mortality rate due to coronary heart disease in populations consuming soy [9]. These studies have suggested that consumption of a traditional Asian diet high in soy may play a pivotal role in prevention of chronic diseases such as atherosclerosis [7]. The atheroprotective effects of soy-based diets have been attributed to its effect on reducing serum cholesterol levels in human nutrition studies [10–14]. Similar findings have also been reported in nonhuman primates fed soy-based diets compared to those fed control diets [15–17]. However, recent studies in the atherosclerosis-susceptible apolipoprotein E knockout (apoE−/−) mouse models [18,19] showed that atherosclerotic lesions are reduced when fed a soy-containing diet despite unchanged serum lipid levels [20–22]. These studies suggest that dietary soy may inhibit atherosclerotic lesion development by mechanism(s) other than lowering serum cholesterol levels. However, the mechanism(s) and component(s) of soy responsible for these effects remain to be resolved.

1.2. Atherosclerosis is a chronic inflammatory disease

Recent studies have unequivocally demonstrated that atherosclerosis is a chronic inflammatory disease [23,24], and vascular inflammation has been identified as an early event in the pathogenesis of atherosclerosis [23,24]. Four early cellular events contribute to

Abbreviations: ApoE−/−, apolipoprotein E knockout; CD54, intercellular adhesion molecule-1; CD62E, E-selectin; CD62P, P-selectin; CD106, vascular cell adhesion molecule-1; CVD, cardiovascular disease; LDL, low-density lipoprotein; MCP-1, monocyte chemoattractant protein-1; oxLDL, oxidized-LDL; PPAR, peroxisome proliferator-activated receptor; TF, tissue factor.

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the initiation of inflammatory processes associated with atherosclerosis: (1) endothelial cell activation resulting in enhanced adhesive property of vascular endothelial cells, (2) activation of monocytes leading to the firm adhesion of circulating inflammatory cells, (3) transmigration of monocytes to the intima where these cells transform to become macrophages, and, finally, (4) uptake of modified low-density lipoprotein (LDL), such as an oxidized form of LDL (oxLDL), by macrophages resulting in transformation of macrophages to lipid-laden macrophages, also known as foam cells (Fig. 1). Hence, the modulation or regulation of the interaction between endothelial and inflammatory cells and the transformation of macrophages to foam cells could be the basis for the beneficial effects of soy-based diets. This review focuses on the atheroprotective effects of soy diets on the four early cellular events contributing to the initiation and progression of atherosclerosis. Since the lipid-lowering effect of soy intake has been discussed previously [25–28], it is not discussed in this review.

1.3. Soy and endothelial cell activation

The cascade of interactions between circulating monocytes and endothelial cells is the prime event in the initiation of atherosclerosis [29,30]. The recruitment of leukocytes, including monocytes, and their adhesion to endothelial cells are a multistep process [29,30] involving initial rolling of monocytes, monocyte activation for subsequent adhesion, and transmigration of monocytes. These steps are controlled by a number of vascular endothelial cell adhesion molecules. Naïve endothelial cells do not promote monocyte adhering to the vascular bed. Initial vascular inflammation results in up-regulation of key vascular endothelial cell adhesion molecules such as E- and P-selectins (referred to as CD62E and CD62P, respectively). Inflamed vascular endothelium expressing CD62E and CD62P results in rolling of monocytes on endothelial cells. Rolling is followed by activation of circulating monocytes. Prior activation is a prerequisite for firm adhesion, the next step in the inflammatory processes. Activation of circulating monocytes could be mediated by a number of pro-inflammatory cytokines, oxLDL, and oxLDL/anti-oxLDL IgG immune complexes. Firm adhesion is regulated by vascular cell adhesion molecule-1 (VCAM-1, CD106) and intercellular adhesion molecule-1 (ICAM-1, CD54). The role of cell adhesion molecules in the initiation of inflammatory process has been demonstrated in studies showing that deletion of both CD62E and CD62P has been shown to reduce the progression of atherosclerosis in apoE−/− mice [31–33]. Importantly, despite having hypercholesterolemic conditions, double knockout mice lacking the expression of apoE−/− and CD106 have been shown to have reduced numbers of atherosclerotic lesions highlighting the critical role of cell adhesion molecules in the initiation and progression of atherogenesis [34]. Collectively, these studies have demonstrated the causal relationship between adhesion of monocytes to endothelial cells as controlling the initial events of atherogenesis. However, studies on the role of soy in regulation of vascular cell adhesion molecules involved in the inflammatory process associated with atherogenesis are only beginning to emerge.

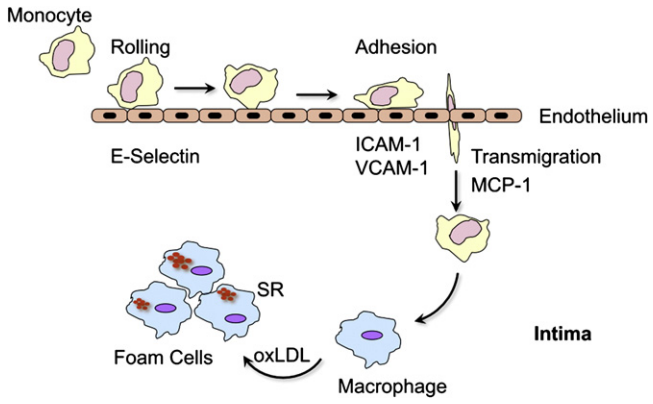

Fig. 1. Inflammatory processes associated with atherosclerosis.

Pro-inflammatory cytokines, TNF-α and IL-1β, have been shown to induce activation of endothelial cells resulting in the coordinated up-regulation of CD54, CD62E and CD106 expression [35–37]. Pretreatment of endothelial cells with 17β-estradiol has been shown to inhibit TNF-α-induced up-regulation of vascular cell adhesion molecules [38]. Studies have shown that soy isoflavones may have phytoestrogenic functions [39], suggesting that soy isoflavones may inhibit vascular endothelial cell adhesion molecule expression. Interestingly, endothelial cells treated with genistein, a principal soy isoflavone (25–50 μM), inhibited TNF-α-induced CD62E and CD106 expression on human umbilical vein endothelial cells and subsequent monocyte adhesion [35,36]. Similar findings were observed using human brain microvascular endothelial cells [40]. An important problem that has not been addressed is whether the *in vitro* findings using pharmacological doses of genistein (50 μM) reflect the *in vivo* effect of soy-based diets or of soy isoflavones. Recently, Chacko et al. [41,42] have demonstrated genistein at a physiologically relevant concentration (1 μM) inhibited monocyte adhesion to endothelial cells in an *in vitro* cell culture model. Furthermore, the authors have also provided evidence that peroxisome proliferator-activated receptor (PPAR)-gamma activation contributes to the genistein-mediated inhibition of monocyte adhesion to endothelial cells in cell culture studies [41,42]. Genistein supplementation (2 g/kg diet) has been shown to up-regulate PPAR-alpha-regulated genes *in vivo* [43]. Earlier work from Shay's group [27,44] has shown that modulation of lipid metabolism by soy isoflavone is in part dependent on PPAR-alpha activation in mice fed low (0.09 g/kg diet) vs. high isoflavone (3 g/kg diet) diets. Furthermore, Dang et al. [45] have provided evidence that genistein (at 1 μM) induces PPAR-gamma activation. Despite these interesting *in vitro* findings, there is no direct evidence that soy-based diets or soy isoflavones regulate the expression of vascular cell adhesion molecules *in vivo*. Preliminary findings from our laboratory have shown that expression of CD54, CD62E and CD106 mRNA levels was about 30% to 50% lower in the descending aorta from soy-fed compared with casein-fed mice (unpublished observation). Based on this premise, it is plausible that the atheroprotective effect of soy-based diets in part could be due to inhibition of inflammation-induced CD54, CD62E and CD106 expression (Fig. 2). However, in-depth *in vivo* or *ex vivo* studies are warranted to convincingly demonstrate the inhibitory effect of soy-based diet on endothelial cell activation associated with chronic inflammatory diseases such as atherosclerosis.

1.4. Soy diet inhibits activation and subsequent adhesion of inflammatory cells

The second step in the inflammatory process is activation and subsequent adhesion of inflammatory cells [23,24]. The adhesive activity of leukocytes is tightly regulated such that circulating monocytes do not adhere to vascular endothelium. However, after activation, the nonadherent monocytes transiently adhere to vascular endothelium. Inflammatory stimuli, such as monocyte chemoattractant protein-1 (MCP-1) and oxLDL, have been shown to activate monocytes, which in turn promote the activated monocytes to firmly adhere to vascular endothelium [46–48]. Moreover, blocking the recruitment of monocytes to sites of inflammation has been shown to reduce lesions and protect animals from atherosclerosis [49,50]. These studies suggest that activation of inflammatory cells is a

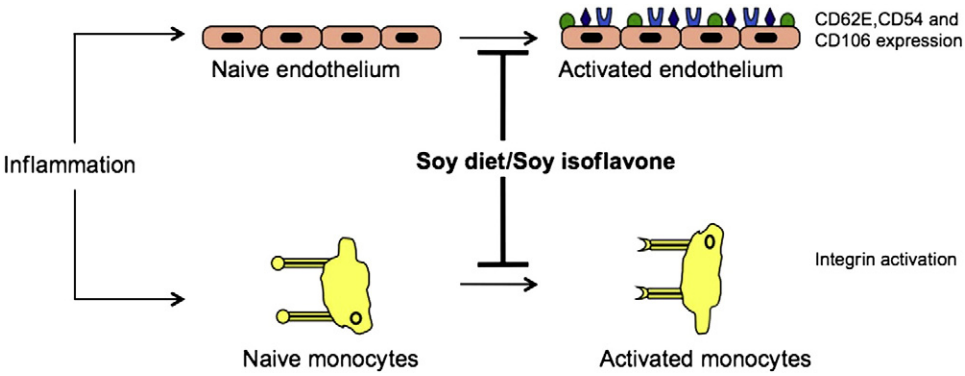

Fig. 2. Soy diet blocks endothelial and monocyte activation.

prerequisite to the progression of the inflammatory processes in atherosclerosis.

Soy feeding has been shown to increase the soy phytochemicals in serum [51,52], suggesting a possibility that the atheroprotective effect of soy isoflavones could also be mediated by blocking inflammatory cell activation and adhesion to vascular endothelium. *In vitro* studies using human monocytic cell lines showed that oxLDL treatment of monocytic cells increases their adhesion to endothelial cells [53]. Furthermore, pre-exposure of monocytes to soy isoflavones (1 μM final concentration with equimolar mixture of genistein, daidzein and equol) as well as to sera from animals fed soy diet inhibited oxLDL-induced monocyte adhesion to endothelial cells [53]. These studies suggest that there is a direct effect of soy isoflavones on monocyte activation and subsequent adhesion, although the detailed mechanism is not known. Based on these findings, it is reasonable to hypothesize that the soy-dependent atheroprotection is mediated through the regulation of monocyte activation (Fig. 2).

1.5. Soy isoflavone regulates monocyte integrin functions

The integrin family of cell surface proteins expressed on inflammatory cells, including monocytes, mediates leukocyte trafficking and adhesion [54,55]. Monocyte integrins on resting cells are not able to bind to their ligands, cell adhesion molecules expressed on vascular endothelium. However, monocyte activation by pro-inflammatory cytokines results in enhanced adhesion of monocytes by regulating the functions of integrins. CD11a, one of the monocyte integrins, is expressed as an inactive low-affinity form [56,57] in circulating (unactivated) monocytes and lymphocytes, and under physiological conditions these cells do not firmly adhere to CD54-expressing vascular endothelial cells. Activation of monocytes and T lymphocytes results in the transformation of CD11a from a low-affinity or avidity to a high-affinity or avidity form [56,57], and such a transformation leads to firm adhesion of inflammatory cells to vascular endothelium through CD11a–CD54 interaction. Recent *in vitro* studies have shown that pre-exposure of monocytes to soy isoflavones (1 μM) inhibited oxLDL-induced monocyte adhesion to endothelial cells [53]. However, this inhibition of monocyte adhesion is not due to the change in CD11a expression. Instead, soy isoflavones inhibited the transformation of low- to high-affinity form of CD11a, as detected using activation epitope-specific anti-CD11a monoclonal antibody [58,59]. These findings suggest that soy isoflavones inhibit the activation-induced transformation of low- to high-affinity form of the CD11a expressed on monocytes. Inhibition of CD11a-dependent monocyte adhesion to endothelial cells by soy isoflavones could be mediated by two possible mechanisms. Tyrosine kinase inhibitors have been shown to inhibit CD11a binding to CD54 [60]; hence genistein, an isoflavone, with kinase-inhibiting property could have contributed to the inhibition of monocyte adhesion. Furthermore, the phytoestrogenic activity of soy isoflavones [61] may be a contributing factor to the inhibition of monocyte adhesion to CD54. This possibility is supported by a recent report by Friedrich et al. [62] showing that monocytes treated with 17β-estradiol poorly adhere to endothelial cells. These probable mechanisms warrant further investigation.

Implications of integrin activation in the inflammatory processes are many. For example, monocyte activation and adhesion to vascular endothelium stimulate the expression of tissue factor (TF) on monocytes [63,64]. Specifically, β1-integrin stimulated monocyte adhesion results in up-regulation of monocyte TF expression [65,66]. TF expression has been shown to increase with progression of human vascular disease [67,68] as well as in animal models [69,70]. In addition, β1-integrin-dependent monocyte adhesion leads to induction of pro-inflammatory cytokines, TNF-α and IL-1β [71,72]. Genistein (20-30 μM) pretreatment has been shown to inhibit β1-integrin-mediated TF, IL-1β and TNF-α [65,71,72] expression, suggesting a possible role for soy isoflavones in preventing the integrin-mediated monocyte activation and subsequent adhesion-dependent inflammatory responses. Collectively, these studies indicate the significance of integrin-mediated monocyte activation in the inflammatory processes associated with atherosclerosis. These findings further suggest that interfering with leukocyte integrin activation may be a novel concept in the identification of anti-inflammatory functions of soy-based diets. However, this concept should be evaluated by determining the expression and activated state of integrins in soy-fed animals using an *ex vivo* monocyte adhesion model.

1.6. Soy diet modulates transmigration of monocytes

Adhesion of inflammatory cells to vascular endothelium is followed by transendothelial migration of these cells to the arterial intima, the third step in the inflammatory processes. Chemokines that drive leukocyte transendothelial migration have been implicated in the initiation and progression of atherosclerosis. These chemokines include MCP-1, RANTES, fractakline, MIP1-α and MIP1-β. Of these chemokines, MCP-1, a member of the CC chemokines, is characterized by its chemokine activity for inflammatory cells, primarily monocytes [49,50]. MCP-1 is secreted locally by inflamed endothelium and activated monocytes [73,74] and is involved in recruitment of monocytes into arterial walls [74]. Furthermore, studies using CC-chemokine receptor 2/apoE double knockout mice have shown that despite having hypercholesterolemic conditions these mice had reduced atherosclerotic lesions [49,50], indicating MCP-1 plays a pivotal role in the inflammatory processes associated with the

pathogenesis and progression of atherosclerosis. Hence, it is reasonable to predict that the atheroprotective effect of soy diet may be mediated by regulating the expression of pro-inflammatory chemokines. Interestingly, soy-based diet inhibits the expression of MCP-1 in the aorta [22], and this observation is also supported by the reduced number of macrophages in atherosclerotic lesion in mice fed soy-based diets compared to control casein diet [22]. Moreover, co-culture of human monocytes with endothelial cells resulted in the increased secretion of pro-inflammatory cytokines, such as IL-6 and IL-8, and the addition of soy isoflavones to the monocyte/endothelial cell co-culture inhibited the secretion of these pro-inflammatory cytokines [53]. However, plasma MCP-1 levels were not different in mice fed soy or control (casein) diet [22]. This finding is also supported by recent studies showing there was no change in the plasma inflammation markers, including MCP-1, in postmenopausal women consuming soy isoflavone-enriched foods compared to placebo controls [75]. These findings suggest that soy diets inhibit pro-inflammatory cytokine and chemokine expression at the local inflammation site of lesion formation rather than at the systemic levels of pro-inflammatory cytokines and chemokines. Based on this principle, the atheroprotective effect of soy-based diets may be mediated in part by inhibiting monocyte migration. Such a possibility could be addressed by determining the monocyte migration by bone marrow chimera approach.

1.7. Soy and foam cell formation

After the transmigration of monocytes into arterial intima, monocytes are transformed to macrophages. The activated macrophages further take up oxLDL generated in the intima [76,77] through its binding to scavenger receptors resulting in lipid-laden macrophages, also known as foam cells [78,79]. This step culminates the initial events of atherogenesis. In addition to promoting foam cells, oxLDL binding to endothelial cells leads to up-regulation of cell adhesion molecules such as CD54 and CD106 [80–82]. Furthermore, oxLDL can induce the expression of MCP-1 by vascular endothelial cells, smooth muscle cells and macrophages [83,84], as well as macrophage colony-stimulating factor [85,86]. MCP-1, a monocyte chemokine, initiates monocytes recruitment, and macrophage colony-stimulating factor promotes differentiation of monocytes into macrophages. Owing to the importance of oxLDL generation and scavenger receptor-mediated uptake of oxLDL by macrophages resulting in foam cell formation, the mechanism by which soy-based diets can prevent atherosclerosis is in part by inhibiting oxLDL generation. Several *in vitro* studies have suggested that LDL oxidation is inhibited by soy isoflavones [87–89]. However, components involved in this effect are not consistent between studies. Soy isoflavones, genistein (2.5 μM) and daidzein have been reported to inhibit copper-induced oxidation of LDL [88,89]. However, Kapiotis et al. [87] have reported that only genistein (2.5 μM), not daidzein, inhibits *in vitro* LDL oxidation. Interestingly, equol and 8-hydroxydaidzein, principal metabolites of daidzein, are potent inhibitors of oxLDL generation than the parent daidzein [89,90]. These *in vitro* studies have also been supported by *in vivo* animal studies. Soy feeding or casein diet supplemented with soy isoflavones (7.3 mg isoflavones/kg diet) has been reported to reduce circulating levels of oxLDL and *ex vivo* LDL oxidation compared with control casein diets in animal models [91,92]. However, clinical studies on the effect of soy isoflavones on oxLDL generation still leave this question unresolved. Following consumption of soy with high isoflavones (210 μM) compared to soy with low isoflavones (7 μM), a decrease in susceptibility of LDL to oxidation has been observed [93]. However, others have reported no effect of soy isoflavones on the generation of oxLDL [94]. The difference could be due to the nature of samples as well as to the nature of assays used to address the antioxidant effects of soy isoflavones. For instance, the study where the isoflavones have no effect on LDL oxidation is based on samples from hypercholesterolemic patients [94], while the protective effects were reported using samples from individuals with normal serum cholesterol levels [93]. Although there are several reports on soy isoflavone on oxLDL generation, there is no report on the effect of soy isoflavones, particularly genistein, on scavenger receptor expression. Recently, soy pinitol, one of the components of soy, has been shown to reduce foam cell formation in human macrophages by inhibiting the expression of scavenger receptors in a cell culture model [95]. However, more detail studies are warranted to confirm and address possible mechanisms by which soy-based diets regulate macrophage scavenger receptor expression.

1.8. Atheroprotective effect of soy peptides

Soy food or soy protein isolate contains two major components with potential bioactivity. The two components are phytochemicals, such as isoflavones associated with soy protein, and the peptides generated from two of the major soy proteins, such as β-conglycinin (or 7S globulins) and glycinin (or 11S globulins). However, the component(s) of soy responsible for its atheroprotective effects is debatable. Mice fed soy protein isolate that was processed to remove phytochemicals (hereafter referred as isoflavone-free soy protein isolate) had lower incidence of atherosclerotic lesions [22]. Attenuated lesions are also associated with a reduced expression of MCP-1 in aorta and a subsequently lower number of macrophages in lesions in isoflavone-free soy protein isolate-fed than in casein-fed apoE−/− mice [22]. Furthermore, the studies of Adams et al. [21,96] have demonstrated that mice fed isoflavone-free soy protein-containing diet, particularly mice fed the β-conglycinin-containing diet, had a pronounced inhibitory effect on the development of atherosclerosis compared to mice fed casein–lactalbumin-based diets. These findings suggest an atheroprotective role for the protein components of soy diet or peptides generated from soy protein such as β-conglycinin and glycinin. Furthermore, these studies suggest that there are bioactive small peptide fractions produced by the digestion of soy protein that are absorbed from the intestinal tract and have favorable effects on preventing atherosclerosis. One of the caveats in animal experiment testing of the atheroprotective effect of soy diets is the use of casein-fed mice as controls. It is possible that the animal-derived proteins may be atherogenic compared to soy- or other vegetable-derived proteins. However, without a vegetable-based protein diet that may be proatherogenic as controls for the animal studies, the debate on any proatherogenic effect of casein will continue. Alternatively, the atheroprotective effect of soy- or other vegetable-derived proteins reemphasizes the concept that consumption of vegetable-derived proteins may have health-promoting effects not observed with the consumption of animal-derived proteins.

In conclusion, atherosclerosis is a chronic inflammatory disease and the cascades of monocyte–endothelial cell interactions and monocyte migration are crucial processes in the development and progression of atherosclerosis. Adhesion of leukocytes, specifically monocyte adhesion to vascular endothelium, and subsequent transmigration of monocytes to intima resulting in the formation of lipid laden foam cells are initial events in the inflammatory processes associated with atherogenesis. In this review, we have discussed possible mechanism(s) by which soy diet or components of soy diet regulate the inflammatory processes associated with atherosclerosis. Specifically, we have discussed how soy or components of soy diet control adhesive functions of vascular endothelium by regulating key endothelial cell adhesion molecules, monocyte integrin function, cytokines and chemokines that control trafficking and migration of monocytes. Finally, we have discussed a possible regulation of oxLDL generation and regulation of scavenger receptor expression by soy

diets. Thus, nutritional intervention by soy or other diets with anti-inflammatory effects could block the inflammatory processes associated with atherogenesis thereby reducing the risk of CVD.

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