SHOSAIKOTO (KAMPO MEDICINE) PROTECTS MACROPHAGE FUNCTION FROM SUPPRESSION BY HYPERCHOLESTEROLEMIA

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The feeding of cholesterol-enriched diet for 2 weeks was enough to reduce nitric oxide (NO), prostaglandin E₂ (PGE₂) and interleukin-1 (IL-1) productions in thioglycollate-elicited murine macrophages. Although not showing anti-hypercholesterolemic action against ICR mice, Shosaikoto, a Kampo medicine, partially prevented the reduction of NO and IL-1 productions induced by the feeding of cholesterol-enriched diet, and completely released the reduction of PGE₂ production. These data suggest that the malfunction of macrophage induced by hypercholesterolemia may contribute to early atherogenesis and that Shosaikoto retains macrophage function to preveny the development of atherosclerosis, even though serum cholesterol is markedly increased.

KEY WORDS hypercholesterolemia; macrophage; Shosaikoto; nitric oxide; prostaglandin E₂; interleukin 1

A critical event in the pathogenesis of atherosclerosis is subendothelial accumulation of lipid-laden foam cells, which are primarily derived from monocytes/macrophages by uptake of oxidatively modified LDL (ox-LDL). Ox-LDL not only leads macrophages to foam cells, but also impairs several macrophage functions. Ox-LDL enhances monocyte chemoattractant protein-1 synthesis and suppresses nitric oxide production and TNF-α and IL-1 gene expression in murine peritoneal macrophages. The oxidation of LDL is considered to occur in the arterial wall, where it is sequestered from circulating antioxidants and contains increased amounts of metal ions. In fact, ox-LDL was detected in human and animal atherosclerotic lesions, but not in circulatory blood. In an in vivo experiment, we have demonstrated that monocytes in hypercholesterolemic rabbits gain adhesion to endothelial cells, and Fan has reported that peritoneal macrophages of hypercholesterolemic rats show enhanced adhesion and chemotactic migration. Shosaikoto, a Kampo medicine which is clinically used to treat chronic hepatitis and cirrhosis, shows anti-atherosclerotic activity in an atherosclerotic rabbits fed a cholesterol-enriched diet. In addition, Shosaikoto enhances uptake and degradation of oxidized LDL by macrophages to prevent cholesteryl ester accumulation and stimulates phagocytic activity and interleukin secretion in macrophages. We therefore attempted to determine the effect of cholesterol-enriched diet on the function of peritoneal macrophage such as NO, PGE₂, IL-1 syntheses and the effect of Shosaikoto on the altered macrophage function in order to investigate the anti-atherosclerotic activity shown by Shosaikoto.

MATERIALS AND METHODS
Male ICR mice (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were used at 7 weeks of age. Hypercholesterolemic mice were fed a 1.25% cholesterol-enriched diet containing 20% milk casein, 50% sucrose, 15% coconut milk, 4.95% crystallized cellulose, 1% corn oil, 5% mineral mixture, 1% vitamin mixture, 1% choline chloride, and 0.3% methionine (Oriental Yeast Co. Ltd., Tokyo, Japan). Macrophages were harvested from the peritoneal cavity 4 days after i.p. injection of 2ml of 3% thioglycollate broth (Difco Laboratories, Detroit, MI). Production of NO, PGE₂ and IL-1 were assayed 24h after LPS challenge (10μg/ml)

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using Griess reagent \(^{10}\) and Elisa kits (Neogen Co., Lexington, KY and PerSeptive Diagnostics, Cambridge, MA), respectively. Shosaikoto (dose per person per day) was prepared as follows. Bupleuri Radix (7g), Pinelliae Tuber (5g), Scutellariae Radix (3g), Ginseng Radix (3g), Zingiberis Rhizoma (4g), Zizyphi Fructus (3g) and Glycyrhizae Radix (3g) were added to 700 ml water, decocted for 1 h and concentrated to 300 ml. This decoction was lyophilized to give 7.2g of powdered extract.

RESULTS AND DISCUSSION

ICR mice were fed a cholesterol-enriched diet for 2 weeks in the absence or presence of treatment with Shosaikoto at a dose of 1.2g/kg of body weight. Although this dose is 10 times higher than that for humans, we chose this dose in this study because our previous studies revealed that the administration of Shosaikoto at this dose had the most prominent effect on macrophage function.\(^{11,12}\) The serum cholesterol concentration in cholesterol-fed mice and those treated with Shosaikoto was not different (245mg/dl and 240mg/dl, respectively), indicating that Shosaikoto does not show anti-hypercholesterolemic action in ICR mice (data not shown). We therefore determined NO, PGE\(_2\), IL-1 syntheses in macrophages 24 h after LPS challenge. The productions of NO, PGE\(_2\) and IL-1 in macrophages prepared from cholesterol-fed mice were markedly reduced compared with macrophages from normal mice (Fig.1). In contrast, Shosaikoto treatment significantly prevented the reduction of NO, PGE\(_2\) and IL-1 syntheses by hypercholesterolemia. The reduction of NO and IL-1 syntheses was partially reduced, whereas that of PGE\(_2\) was completely prevented. On the other hand, Shosaikoto itself stimulates NO and PGE\(_2\) syntheses in normal macrophages and showed a tendency to stimulate IL-1 synthesis.

![Graph](image_url)

**Fig.1.** Effect of Shosaikoto on (a) NO, (b) PGE\(_2\) and (c) IL-1 Productions by Macrophages Derived from Cholesterol-Fed Mice

Macrophages were prepared from normal mice or mice fed a cholesterol-enriched diet in the absence or presence of oral treatment with Shosaikoto (1.2g/kg/day) for 2 weeks. NO, PGE\(_2\) and IL-1 productions were determined as described in MATERIALS and METHODS. ■ normal group; ■, cholesterol group; ■, Shosaikoto group; ■, cholesterol/Shosaikoto group. Values indicate the net amount which was synthesized for 24 h after LPS challenge and represent means ± S.E. of 5 mice. **p<0.01, *p<0.05.

The inhibition of NO synthesis and IL-1 gene expression in macrophages by oxidized LDL and the inhibition of PG synthesis by acetylated LDL have been reported in vitro studies.\(^{4,6,14}\) In this study, we first indicated that a short period of hypercholesterolemia was sufficient to modify macrophage function, that is, to reduce NO, PGE\(_2\) and IL-1 syntheses. NO derived from endothelial cells was known to play a critical role in the regulation of atherogenesis by inhibiting platelet adhesion and aggregation,\(^{15}\) smooth muscle proliferation,\(^{16}\) and endothelial cell-leukocyte interaction\(^{17}\). In contrast, NO produced by macrophages is considered to be necessary for cytolytic action against infected bacteria, while its implication for the regulation of atherogenesis is still unclear. However, long-term inhibition of NO synthesis promotes atherosclerosis\(^{18}\) and the administration of L-arginine, which was a substrate of NO synthase, reduces atherogenesis,\(^{19}\) suggesting that NO plays a role in anti-atherogenesis and further NO produced by macrophages is also likely to be
implicated. On the other hand, many biological activities of PG and IL-1 were extensively investigated, and PGE$_2$ and IL-1 were found to regulate immune response negatively and positively, respectively, resulting in the concerted modulation of systemic immunity. Since immunosuppression is known to enhance atherosclerosis,\textsuperscript{20,21} the reduction of PGE$_2$ and IL-1 syntheses by hypercholesterolemia could lead to the disturbance of immune response, affecting the development of atherosclerosis. In this study, the suppression of macrophage function was observed only 2 weeks after cholesterol feeding. If this alteration is mediated by ox-LDL or minimally ox-LDL, oxidation of LDL would occur rapidly in blood vessels, although ox-LDL is usually detected in or near atherosclerotic lesions, but not in normal blood vessels. Other possibilities are that cholesterol accumulation by the uptake of native LDL through apoB/E receptor could be sufficient to suppress macrophage function or that an unknown suppressor of macrophage was produced fairly early after cholesterol-feeding. Although the earliest event in atherosclerosis is considered to be the adhesion of monocytes to endothelial cells or the accumulation of ox-LDL by macrophages, the malfunction of macrophage caused by hypercholesterolemia may precede the events so far demonstrated, resulting in the initiation of further atherogenesis. Considering the mechanism by which Shosaikoto releases the suppression of macrophage function by hypercholesterolemia, it is conceivable that Shosaikoto inhibits the production of a substance that suppresses macrophage function or stimulates macrophage regardless of the presence or absence of the suppressor of macrophage function. Conclusively, Shosaikoto protects macrophage function from hypercholesterolemia, suggesting that this action may be related to anti-atherosclerotic action shown by Shosaikoto.

REFERENCES


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