Increase of Vitamin E Content in LDL and Reduction of Atherosclerosis in Cholesterol-Fed Rabbits by a Water-Soluble Antioxidant-Rich Fraction of Salvia miltiorrhiza

Yih-Jer Wu, Chuang-Ye Hong, Shing-Jong Lin, Paulin Wu, Ming-Shi Shiao

Abstract—Antioxidants that prevent LDL from oxidation may reduce atherosclerosis. Salvia miltiorrhiza Bunge is a Chinese herb widely used for the treatment of atherosclerosis-related disorders. Salvianolic acid B (Sal B), a water-soluble polyphenolic antioxidant isolated from the roots of this plant, was found to scavenge 1,1-diphenyl-2-picrylhydrazyl radicals and inhibit LDL oxidation more effectively than probucol. In order to evaluate the antiatherogenic potential, New Zealand White rabbits were fed for 12 weeks a normal diet, a high cholesterol diet, a high cholesterol diet containing 1% probucol, or a high cholesterol diet containing a 5% water-soluble extract of S miltiorrhiza (SM). Both SM and probucol feeding reduced plasma cholesterol. LDLs from the SM-treated group were more resistant to Cu²⁺-induced oxidation and contained more vitamin E (21.7±2.1 nmol/μmol LDL cholesterol) than did LDLs from the high cholesterol diet group (9.6±1.8 nmol/μmol LDL cholesterol) (P<.005). Endothelial damage, determined at week 6, was reduced by 53% in the SM group (P<.001). SM treatment reduced the atherosclerotic area in the abdominal aorta by 56% (P<.005) and cholesterol deposition in the thoracic aorta by 50% (P<.005). The severity of atherosclerosis in the SM group was significantly reduced after adjustment by using cholesterol exposure as an index of the cholesterol-lowering effect. This study concludes that the reduction of atherosclerosis by SM relies not only on its cholesterol-lowering effect but more heavily on its antioxidant potential to prevent endothelial damage and inhibit LDL oxidative modification in hypercholesterolemic animals. (Arterioscler Thromb Vasc Biol. 1998;18:481-486.)

Key Words: Salvia miltiorrhiza | salvianolic acid B | vitamin E | atherosclerosis | oxidized LDL

The pathogenesis of atherosclerosis involves endothelium dysfunction, infiltration of monocytes, activation of monocytes into macrophages, and smooth muscle cell proliferation. Recent studies have demonstrated that OxLDL plays an important role in the initiation and progression of atherosclerosis. Minimally modified LDL is capable of inducing gene expression in endothelial cells that may result in the acceleration of atherogenesis. On further modification in the intima, OxLDL is taken up by scavenger receptors of macrophages, gradually leading to the formation of foam cells and fibrous plaques. Immunochemical studies have demonstrated that OxLDL is present in the atherosclerotic lesions of animals and humans.

Antioxidants that prevent LDL from oxidative damage may interrupt the progression of atherosclerosis. Vitamins E and C protect LDL from oxidative damage in vitro and decrease the morbidity of coronary heart disease. Probucol inhibits atherosclerosis in nonhuman primates and rabbits. Recently, N,N′-diphenyl-phenylenediamine has been demonstrated to reduce atherosclerosis in cholesterol-fed rabbits and apoE-deficient mice. The ability of lipophilic antioxidants to reduce atherosclerosis and coronary heart disease is mainly due to the protection of LDL from oxidative modification. Since water-soluble antioxidants are unable to adhere to LDL particles, they are unable to protect LDL from oxidation in the subendothelial space. However, several studies suggest that water-soluble antioxidants, such as vitamin C, are effective in inhibiting LDL oxidation by the preservation of endogenous antioxidants in LDL. Recent studies have also shown that flavonoids, a class of water-soluble antioxidants, are useful in reducing the risk of coronary heart disease. Glabridin, a polyphenolic compound with medium polarity from licorice, protects LDL against lipid peroxidation in humans and reduces atherosclerotic lesion areas in apoE-deficient mice.

Salvia miltiorrhiza Bunge (Labiatae), an eminent herb in the treatment of cardiovascular disorder (called blood stasis in traditional Chinese medicine), is widely used in China, Japan, and Taiwan. Its nonpolar extracts contain tanshinones, which can inhibit platelet aggregation and protect myocardium against ischemia-induced derangement. The aqueous extract of S miltiorrhiza contains phenolic compounds that are effective
in protecting liver microsomes, hepatocytes, and erythrocytes against oxidative damage. Among them, Sal B (see Fig 1) is a potent hepatoprotective agent and water-soluble antioxidant.

The purpose of the present study is to elucidate the potential of Sal B in the inhibition LDL oxidation and of a Sal B–rich fraction of S. miltiorrhiza (SM) in the reduction of the severity of atherosclerosis in hypercholesterolemic rabbits.

**Methods**

**Materials and Animals**

Probucol was obtained from Marion Merrell Dow. Caffeic acid, 1,1,3,3-tetramethoxypropane, DPPH, BSA, α-tocopherol, retinyl acetate, Evans blue, Sudan IV, and hematoxylin were purchased from Sigma Chemical Co. Sal B was a gift from the Institute of Materia Medica, Chinese Academy of Medical Sciences at Beijing. Male NZW rabbits were obtained from the Animal Center of National Taiwan University. High fiber rabbit chow (as normal diet) was purchased from Purina.

**Preparation of SM and Determination of Sal B Content**

The dry roots of S. miltiorrhiza were extracted with a mixture of water and ethanol (4:1, vol/vol) at room temperature for 24 hours. After evaporation of solvent under reduced pressure, the extract (SM) was stored under nitrogen at 4°C before use. The content of Sal B in SM was determined by reversed-phase HPLC. The column (5C18, 4.6×250 mm) was eluted with 25% aqueous methanol at 1.0 mL/min for 6 minutes. The volume percentage of methanol was increased to 40% linearly in the next 5 minutes and kept constant for 20 minutes. During the entire elution, a low percentage of acetic acid was added to suppress ionization of phenolic acids. Sal B was eluted at a retention time of 24.9 minutes. Quantification of Sal B was based on peak area at 290 nm.

**DPPH Radical Scavenging and Inhibition of LDL Oxidation**

The DPPH radical–scavenging activities of Sal B, SM, and probucol were determined. In brief, 1 vol of acetate buffer (100 mmol/L, pH 5.5), 1 vol of ethanol, and 0.5 vol of freshly prepared DPPH ethanolic solution (500 mmol/L) were mixed. After adding the test compound, the mixture was incubated at 25°C for 90 minutes. The change in absorption at 517 nm was then determined.

Fasting plasma samples were collected from healthy male adults not given vitamin supplements. LDL was obtained by ultracentrifugation from a density range of 1.019<d<1.063 (adjusted by NaBr). LDL was extensively dialyzed against PBS (5 mmol/L phosphate buffer and 145 mmol/L NaCl, pH 7.4) at 4°C under nitrogen for 24 hours. In vitro LDL oxidation was carried out in a 96-well microtiter plate at 37°C. Probucol stock solution (2.0 mmol/L) was prepared in ethanol. It was diluted to 50 μmol/L into 10% aqueous ethanol before use as a positive control. Sal B and SM were dissolved in PBS. A 50 μL aliquot of LDL (0.9 mg cholesterol per milliliter) in each well was preincubated with test compound for 1 hour. The final volume in each well was adjusted to 100 μL with PBS. LDL oxidation was initiated by adding CuSO4 to a final concentration of 10 μmol/L. After incubation, 150 μL EDTA (2 mmol/L) was added. A 100-μL portion of the mixture was then transferred to a microvial containing 0.9 mL of 2-propanol. The precipitates were removed by centrifugation. The concentration of conjugated dienes in the supernatant was determined by absorption at 234 nm.

**Animal Treatment**

Male NZW rabbits were randomly divided into four groups. Five rabbits in the control group were fed a normal diet. Seven rabbits in the high-cholesterol group were fed a high-cholesterol diet (normal diet supplemented with 1% cholesterol and 4% corn oil). Seven rabbits in the probucol group were fed a high-cholesterol diet plus 1% (wt/wt) probucol. Seven rabbits in the SM group were fed a high-cholesterol diet plus 5% (wt/wt) SM. The selection of SM dose was based on its antioxidant activity comparable to that of probucol in Cu2+-induced LDL oxidation (see Table 1). Diets and drinking water were provided ad libitum. Animals were bled periodically for measurement of plasma cholesterol and liver function. During the 12-week feeding period, we adhered to the guidelines for care and use of laboratory animals.

**Plasma Cholesterol and Lipoprotein Analysis**

Fasting plasma levels were obtained from animals after 0, 1, 3, 6, 9, and 12 weeks of feeding. Concentrations of plasma and lipoprotein cholesterol were determined enzymatically. Cholesterol exposure was calculated as the area under the curve of plasma cholesterol versus time. For lipoprotein analysis, an aliquot of plasma was preincubated with Sudan black for 20 minutes. Electrophoresis was performed by using polyacrylamide gel (Sebia) with a discontinuous gradient (2% in the upper layer and 3% in the lower layer).

**Susceptibility of LDL Oxidation Ex Vivo**

Fasting blood samples were collected into evacuated tubes (K-, EDTA) from rabbits after 12 weeks of feeding. The LDL fraction (1.019<d<1.063) was collected by ultracentrifugation and extensively dialyzed in PBS (pH 7.4). The concentration of dialyzed LDL was adjusted to 0.9 mg cholesterol per milliliter. It was incubated with 5.5), 1 vol of ethanol, and 0.5 vol of freshly prepared DPPH ethanolic solution (500 mmol/L) were mixed. After adding the test compound, the mixture was incubated at 25°C for 90 minutes. The change in absorption at 517 nm was then determined.

| Table 1. IC50 Values and Relative Potency of Probucol, Sal B, and SM |
|-----------------|-----------------|-----------------|-----------------|
|                 | DPPH Scavenging | Inhibition of LDL Oxidation |
|                 | IC50, μg/mL     | Relative Potency | IC50, μg/mL     | Relative Potency |
| Probucol        | 53              | 1.0             | 2.79            | 1.0             |
| Sal B           | 12              | 4.4             | 0.85            | 3.3             |
| SM              | 200             | 0.27            | 14.4            | 0.19            |

IC50 values were obtained from the concentration–response curves. For comparison, the potency of probucol was set at 1.0. When expressed on a molar basis, the IC50 values in the inhibition of LDL oxidation by Sal B (formula weight, 718 g/mol) and probucol were 1.2 and 5.4 μmol/L, respectively. Data represent mean values of three determinations.

**Selected Abbreviations and Acronyms**

ALT = alanine aminotransferase  
AST = aspartate aminotransferase  
BUN = blood urea nitrogen  
DPPH = 1,1-diphenyl-2-picrylhydrazyl  
EBA = Evans blue–albumin  
HPLC = high-performance liquid chromatography  
NZW = New Zealand White  
OxLDL = oxidized LDL  
Sal B = salvianolic acid B  
SM = Salvia miltiorrhiza extract  
TBA = thiobarbituric acid  
TBARS = TBA-reactive substance
CuSO\(_4\) (final concentration, 25 \(\mu\)mol/L) at 37°C. A subsequent assay was performed according to the procedure of Puhl et al\(^{11}\) with modification. Formation of malondialdehyde was determined as TBARS.\(^{26}\) In brief, an aliquot of incubation solution was added with TBA solution (0.6%, wt/vol) and heated at 95°C for 45 minutes. After centrifugation, TBARS in the supernatant was measured by absorption at 532 nm.

**Vitamin E Content in LDL**

Without dialysis, a 200 \(\mu\)L aliquot of LDL was added with an equal volume of ethanol containing 7.5 \(\mu\)mol/L retinyl acetate as an internal standard. The mixture was extracted with \(n\)-hexane containing BHT (0.4 mg/mL) in darkness. The hexane layer (1.5 mL) was dried under a stream of nitrogen, and the residues were resuspended in 100 \(\mu\)L mobile phase (acetonitrile/ethanol/water, 70:30:30, vol/vol). After filtration through glass wool, the content of vitamin E (as \(\alpha\)-tocopherol) was determined by reversed-phase HPLC.\(^{32}\)

**Endothelial Damage**

Endothelial damage was determined in separate animals after 6 weeks of an identical feeding schedule. Quantification was based on the leakage of EBA conjugate as a macromolecular tracer.\(^{33}\) Animals were anesthetized by a mixture of ketamine and xylazine hydrochloride solution. The right femoral artery, vein, and right carotid artery were cannulated. Fifteen milliliters of EBA solution (200 mg BSA per milliliter) was slowly injected into the femoral vein. Exactly 5 minutes after injection, an overdose of pentobarbital was given. Shortly before euthanasia of the animal, heparin (5000 USP units) was injected after injection, an overdose of pentobarbital was given. Shortly before euthanasia of the animal, heparin (5000 USP units) was injected intravenously through the femoral vein to prevent intravascular blood coagulation. The arterial system was perfused immediately with heparinized saline (2 U/mL) via the right carotid artery at a pressure of 120 mm Hg. After the fluid was drained from the femoral artery and the vein turned clear, the perfusate was switched to a neutral-buffered 10% formalin solution. Perfusion was continued at the same pressure for another 10 minutes as a preliminary perfusion fixation. For further fixation, the thoracic aorta was excised and immersed in 10% formalin for 1 hour. The adventitial tissue and branches were carefully removed. Each aortic specimen was cut longitudinally and dissected into six pieces (100 mm\(^2\) per segment). The specimens were stained with Harris’ hematoxylin for 1 minute. An epifluorescence microscope was used for detection and quantification of EBA leaky foci. The EBA fluorescence was examined with green excitation at 546 nm.

**Extent of Atherosclerosis and Cholesterol Deposition**

After 12 weeks of feeding, the animals were killed, and the aortas, from aortic arch to iliac bifurcation, were collected and cleaned of adhering tissue. Abdominal aortas were stained with a solution of Sudan IV to visualize the lesion area.\(^{17}\) Sudanophilic areas were photographed and measured by computer-assisted planimetry. The differences in number of EBA leaky foci between groups were analyzed by paired t-test. The other statistical analyses were obtained by using unpaired t test or ANOVA. A value of \(P<0.05\) was considered statistically significant.

**Results**

**DPPH Scavenging and Inhibition of LDL Oxidation by Sal B**

The content of Sal B in SM was 4.44%, as determined by reversed-phase HPLC. There was no detectable vitamin E, C, or \(\beta\)-carotene in the SM fraction. Both SM and Sal B exhibited free radical–scavenging activity in DPPH assay and were effective in preventing Cu\(^{2+}\)-induced LDL oxidation (see Table 1). On the basis of IC\(_{50}\) values, Sal B was 4.4 times more potent than probucol in DPPH assay and 3.3 times more potent than probucol in Cu\(^{2+}\)-induced LDL oxidation assay. It was estimated that Sal B accounted for up to 75% of antioxidant activity in SM, since Sal B was 17 times more potent than SM in both assay systems.

There was no detectable change in wavelength (\(\lambda_{\text{max}}\)) or molecular absorption (log \(e\)) of Sal B at 50 \(\mu\)mol/L when incubated with LDL (100 \(\mu\)g/mL) in PBS: \(\lambda_{\text{max}}\) (log \(e\)) = 289 (4.16), 310 (4.10), and 330 (4.05). There was no indication of direct binding of Sal B with LDL particles in solution. Similar observations were also found by using fresh, extensively dialyzed human LDL preparations.

**Animals**

There were no differences in body weights at the end of the 12-week feeding period (normal, 3.0±0.2 kg; high cholesterol, 3.1±0.3 kg; probucol, 2.9±0.2 kg; and SM, 3.1±0.2 kg). Hypertriglyceridemia and hyperglycemia occurred in all groups of animals fed a high cholesterol diet; neither probucol nor SM significantly prevented the elevation of plasma triglyceride and glucose. Probucol treatment caused an elevation of AST values (normal, 25±3 U/L; high cholesterol, 36±5 U/L; and probucol, 59±9 U/L). The SM group had an AST value (10±4 U/L) lower than that of the high cholesterol group (\(P<0.05\)).

There were no significant differences in ALT, electrolyte, BUN, or creatinine values among the three cholesterol-fed groups. SM treatment did not cause any additional abnormality, as indicated by the biochemical parameters.

**Plasma Cholesterol and Aortic Cholesterol Exposure**

Plasma cholesterol levels were increased during the feeding period in rabbits fed a high cholesterol diet. SM and probucol treatment attenuated the increase in plasma cholesterol (see Fig 2). Cholesterol exposure, defined as the area under the curve of plasma cholesterol versus time, was determined to estimate the effect of cholesterol lowering on atherosclerosis reduction. SM caused a 29% decrease and probucol caused a 44% decrease in cholesterol exposure compared with the high cholesterol group. Reduction of plasma cholesterol by SM and probucol occurred predominantly in \(\beta\)-VLDL (\(d<1.019\)) (see Fig 3). Probucol treatment decreased HDL cholesterol levels at weeks 6 and 12 compared with levels in the high cholesterol group (\(P<0.005\)) and levels before treatment (week 0 versus week 12, \(P<0.0001\)). Throughout the feeding, no difference in HDL cholesterol between the SM and high cholesterol groups was observed.

**Oxidative Susceptibility and Vitamin E Content in LDL**

LDLs collected from SM- and probucol-treated groups were more resistant to oxidation than those from the high chole-
terol group (see Fig 4). Time required to achieve half-maximum TBARS production was defined as antioxidativity. The antioxidativity of probucol (34.8±1.1 hours) was the longest among the three cholesterol-fed groups. The antioxidativity of the SM-treated group (6.2±0.2 hours) was significantly higher than that of the high cholesterol group (1.1±0.1 hour).

**Figure 3.** Cholesterol contents in lipoprotein fractions corresponding to VLDL (d<1.019) (A), LDL (1.019<d<1.063) (B), and HDL (1.063<d<1.210) (C). Hatched bar indicates normal group (n=5); solid bar, high cholesterol (high Chol) group (n=7); cross-hatched bar, probucol group (n=7); and open bar, SM group (n=7). *P<.05 and †P<.005 vs time-matched high Chol group in panel A. *P<.05 vs probucol group in panel C.

**Figure 4.** Time course of Cu²⁺-induced LDL oxidation. TBARS values were measured by absorption at 532 nm and expressed as nanomoles malonaldehyde per milligram LDL cholesterol (nmol/mg chol). Groups are as follows: high cholesterol (n=7, ●), probucol (n=7, ○), and SM (n=7, ▲). Each data point represents mean±SEM of an individual group.

Cholesterol feeding decreased vitamin E content in LDLs. In the high cholesterol group, the value (9.6±1.8 nmol/μmol cholesterol) was 32% less than that in the normal group (14.1±1.6 nmol/μmol cholesterol) (P<.05) (see Table 2). Probucol treatment further decreased vitamin E content in LDLs by 15%. Vitamin E content in LDLs collected from the SM-treated group was 21.7±2.1 nmol/μmol cholesterol, a value 2.3-fold higher than that in the high cholesterol group (P<.005).

**Protection of Endothelial Damage**
Endothelial damage, as determined by the number of EBA leaky foci in the thoracic aortas, was increased by cholesterol feeding. The number of leaky foci was significantly increased in the high cholesterol group (4.10±0.95 foci per square millimeter) compared with the normal group (0.84±0.03 foci per square millimeter) (P<.01) (see Table 3). Compared with the high cholesterol group, endothelial damage was reduced by 42% in the SM group (P<.01) and by 34% in the probucol-treated group (P<.05). To reveal the protective effect of SM against endothelial damage caused by cholesterol feeding, the number of leaky foci was corrected by subtracting the basal value of the normal group from the treatment group value. SM treatment decreased cholesterol feeding–induced endothelial damage by 53% (P<.05).

**Reduction of Atherosclerotic Area and Cholesterol Deposition**
Severe atherosclerosis was developed in animals after 12 weeks of a high cholesterol diet. The atherosclerotic area in the

**TABLE 2. Plasma Cholesterol, LDL Cholesterol, and Vitamin E Content in LDL of Rabbits Treated for 12 Weeks**

<table>
<thead>
<tr>
<th></th>
<th>Normal (n=5)</th>
<th>High Cholesterol (n=7)</th>
<th>Probucol (n=7)</th>
<th>SM (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma cholesterol</td>
<td>1.1±0.1</td>
<td>74.1±6.4*</td>
<td>39.5±4.2</td>
<td>63.0±4.6</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.32±0.21</td>
<td>11.4±1.4</td>
<td>10.2±2.8</td>
<td>9.7±1.5</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>14.1±1.6</td>
<td>9.6±1.8†</td>
<td>8.1±1.0†</td>
<td>21.7±2.1</td>
</tr>
</tbody>
</table>

Plasma cholesterol and LDL cholesterol were expressed as millimolar values. The vitamin E content in LDL was expressed as nanomoles α-tocopherol per micromole LDL cholesterol. The value was 13.4±1.1 nmol α-tocopherol per micromole LDL cholesterol before the animals were divided for the feeding experiment. Data represent mean±SEM of an individual group.

*P<.05 vs corresponding probucol group; †P<.005 vs corresponding SM group.
abdominal aorta was 37.2 ± 4.4% and the cholesterol deposition in the thoracic aorta was 28.7 ± 1.9 mg/g in the high cholesterol group (see Fig 5). Probucol treatment resulted in a 74% decrease in atherosclerotic area and a 56% decrease in cholesterol deposition. SM treatment caused a 56% decrease in atherosclerotic area and a 50% decrease in cholesterol deposition. Both decreases were statistically significant (P<.005) compared with the high cholesterol group values.

**Table 3. Endothelial Damage Determined by Leakage of EBA**

<table>
<thead>
<tr>
<th>Segment</th>
<th>Normal</th>
<th>High Cholesterol</th>
<th>Probucol</th>
<th>SM</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0.75</td>
<td>2.19 (1.44)</td>
<td>0.80 (0.05)</td>
<td>1.11 (0.36)</td>
</tr>
<tr>
<td>S2</td>
<td>0.76</td>
<td>2.31 (1.55)</td>
<td>1.75 (0.99)</td>
<td>2.21 (1.45)</td>
</tr>
<tr>
<td>S3</td>
<td>0.87</td>
<td>3.31 (2.44)</td>
<td>2.23 (1.36)</td>
<td>2.23 (1.36)</td>
</tr>
<tr>
<td>S4</td>
<td>0.86</td>
<td>3.62 (2.76)</td>
<td>2.83 (1.97)</td>
<td>2.34 (1.48)</td>
</tr>
<tr>
<td>S5</td>
<td>0.91</td>
<td>4.74 (3.83)</td>
<td>2.49 (1.58)</td>
<td>2.59 (1.68)</td>
</tr>
<tr>
<td>S6</td>
<td>0.86</td>
<td>8.44 (7.58)</td>
<td>6.03 (5.17)</td>
<td>3.68 (2.82)</td>
</tr>
</tbody>
</table>

Mean ± SEM

Endothelial damage was determined after 6 weeks of feeding. S1–S6 denote the segments (100 mm² per segment) of thoracic aorta from the distal end (diaphragm) to the proximal end (ligamentum arteriosum). Values in parentheses indicate the corrected numbers of leaky foci obtained after subtracting the corresponding values of the normal group from the treatment group. Paired t tests were used for statistical analysis.

*Vs high cholesterol group.

Discussion

The present study showed that water-soluble SM, which contained Sal B as a potent antioxidant, scavenged DPPH radicals and inhibited Cu²⁺-induced LDL oxidation. SM feeding reduced endothelial damage and the severity of atherosclerosis in cholesterol-fed NZW rabbits. LDLs from SM-treated animals contained more vitamin E and were more resistant to oxidation ex vivo.

The content of SM in a high cholesterol diet was 5.0%. Since SM contained only 4.4% of Sal B, the actual concentration of Sal B in the chow was only 0.22%, a value much lower than that of probucol (1%). The DPPH-scavenging activity of a water-soluble antioxidant was dependent on its oxidizable functional group. Sal B and SM exerted radical-scavenging activities effectively in the DPPH system. However, antioxidant activity tested in the LDL system was further dependent on the interaction with LDL particles. Lipophilic antioxidants, such as probucol, bound to LDL and augmented the protective effects in LDL oxidation. It is interesting to notice that the relative potency of Sal B to SM remained unchanged in both assay systems. This is an indirect evidence that the antioxidant activity of SM predominantly came from Sal B. Being water-soluble and negatively charged, Sal B is less likely to bind to LDL through the Cu²⁺ binding sites. At a 40-fold higher concentration than its IC₅₀ value, Sal B did not exhibit a detectable wavelength shift or absorption change when coincubated with LDL from the high cholesterol group (authors’ unpublished data, 1997).

The severity of the atherosclerotic area in the abdominal aorta foretold the severity of cholesterol deposition in the thoracic aorta, and vice versa. There was a linear correlation (γ=1.224x−1.54, r²=.807, P<.001) between atherosclerotic area and cholesterol deposition. Cholesterol exposure is a known risk factor of atherosclerosis in cholesterol-fed rabbits. To reveal the influence of factors other than elevation of plasma cholesterol, the atherosclerotic area was normalized by cholesterol exposure. Results showed that SM and probucol significantly reduced the atherosclerotic area per unit of cholesterol exposure. Atherosclerotic areas under similar amounts of cholesterol exposure were less in the SM-treated (−40%) and probucol-treated (−53%) groups than in the high cholesterol group. It is concluded that the antiatherosclerotic effects of SM and probucol rely not only on the cholesterol-lowering effects but also on their antioxidant activities.

Endothelial dysfunction is an early event in the pathogenesis of atherogenesis. The present study demonstrated that SM treatment significantly reduced endothelial damage. Antioxidants, including vitamin E and probucol, are reported to restore endothelial function in cholesterol-fed rabbits. This suggests that Sal B in SM may, on one hand, scavenge free radicals in the bloodstream and reduce their direct injury to aortic endothelium in hypercholesterolemic rabbits. On the other hand, the role of Sal B in resisting LDL oxidation in the subendothelial space could be the result of the preservation of vitamin E in LDL particles in the circulation. It has been reported that vitamin C, but not probucol, preserves lipophilic antioxidants in LDL during oxidative modification. A recent study in humans also indicates that probucol decreases serum concentrations of diet-derived lipophilic antioxidants,
including vitamin E. Since the vitamin E content in LDL was higher in the SM-treated group than in the high cholesterol group and since the SM fraction did not contain vitamin E or C, we conclude that Sil B in SM may indirectly protect LDL from oxidative modification by scavenging free radicals in the bloodstream of cholesterol-fed rabbits and sparing the vitamin E in LDL from being oxidized.

Acknowledgments

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