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*<.01). 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 antiatherogenic 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. Further modification in the intima, OxLDL is taken up by the 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 \textit{Salvia 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, \( \alpha \)-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. Female 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 \textit{Salvia 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 35% linearly in the next 4 minutes and maintained isocratically for 5 minutes. It was further 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 37°C for 90 minutes. The change in absorbance 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 CuSO\(_4\) 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 minivial containing 0.9 mL of 2-propanol. The precipitates were removed by centrifugation. The concentration of conjugated dienes in the supernatant was determined by absorbance 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 Cu\(^{2+}\)-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 prestained 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\(_3\)-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

**TABLE 1. IC\(_{50}\) Values and Relative Potency of Probucol, Sal B, and SM**

<table>
<thead>
<tr>
<th></th>
<th>DPPH Scavenging IC(_{50}), μg/mL</th>
<th>Relative Potency</th>
<th>Inhibition of LDL Oxidation IC(_{50}), μg/mL</th>
<th>Relative Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probucol</td>
<td>53</td>
<td>1.0</td>
<td>2.79</td>
<td>1.0</td>
</tr>
<tr>
<td>Sal B</td>
<td>12</td>
<td>4.4</td>
<td>0.85</td>
<td>3.3</td>
</tr>
<tr>
<td>SM</td>
<td>200</td>
<td>0.27</td>
<td>14.4</td>
<td>0.19</td>
</tr>
</tbody>
</table>

IC\(_{50}\) 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 IC\(_{50}\) 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.
CuSO₄ (final concentration, 25 μmol/L) at 37°C. A subsequent assay was performed according to the procedure of Puhl et al.²⁹ with modification. Formation of malonaldehyde was determined as TBARS.²⁹ 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 μL aliquot of LDL was added with an equal volume of ethanol containing 7.5 μmol/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 μL mobile phase (acetonitrile/ethanol:water:acetic acid:water:0.001% TFA, 70:30:30:1:1, vol/vol). After filtration through glass wool, the content of vitamin E (as α-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 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 solution. The right femoral artery, vein, and right carotid artery were coagulated. The adventitial tissue and branches were carefully removed. Each aortic specimen was cut longitudinally and dissected into six pieces (100 mm² 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.³⁷ Sudanophilic areas 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.³⁷ Sudanophilic areas were photographed and measured by computer-assisted planimetry. Thrombogenic aortas were weighed, minced, and extracted with a mixture of chloroform and methanol (2:1, vol/vol) for total lipids. Aliquots of the lipid extracts were saponified in an ethanolic KOH solution. Cholesterol was extracted into n-hexane, and levels were determined by using a microscale method of Abell et al.³⁴

**Biochemical Measurement**

Blood samples were collected before and 1 and 12 weeks after feeding for measurement of plasma Na⁺, K⁺, Cl⁻, triglyceride, glucose, BUN, creatinine, AST, and ALT. For the SM group, additional blood analysis was carried out after 1 week of feeding for evaluation of acute toxicity.

**Statistical Analysis**

Results were reported as mean±SEM. 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<.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 β-carotene in the SM fraction. Both SM and Sal B exhibited free radical–scavenging activity in DPPH assay and were effective in preventing Cu²⁺-induced LDL oxidation (see Table 1). On the basis of IC₅₀ values, Sal B was 4.4 times more potent than probucol in DPPH assay and 3.3 times more potent than probucol in Cu²⁺-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 (λₑₓ) or molecular absorption (logε) of Sal B at 50 μmol/L when coincubated with LDL (100 μg/mL) in PBS: λₑₓ (logε)=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<.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 β-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<.005) and levels before treatment (week 0 versus week 12, P<.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 choles-
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 antioxi-
dativity of the SM-treated group (6.2±0.2 hours) was signif-
icantly higher than that of the high cholesterol group (1.1±0.1
hour).

Figure 2. Plasma cholesterol (Chol) concentration (A) and Chol
exposure (B). Groups are as follows: normal (n=5, □), high Chol
(n=7, △), probucol (n=7, ○), and SM (n=7, ▲). Plasma Chol
concentrations were lower in SM- and probucol-treated groups
compared with the high Chol group (two-way ANOVA, *P<.005).
†P<.05 vs high Chol in panel A. Chol exposure was defined as
the area under the curve of plasma Chol vs time. *P<.05 and
†P<.005 vs high Chol; ‡ P<.05 vs time-matched probucol group, and
‡P<.005 vs time-matched high Chol group in panel B.

Figure 3. Cholesterol contents in lipoprotein fractions corre-
spanding 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<.0005 vs time-matched high Chol group
in panel A. *P<.05 vs time-matched probucol group, and
†P<.0001 vs 12-week 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 repre-
sents mean±SEM of an individual group.

Cholesterol feeding decreased vitamin E content in LDLs.
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±1.6 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.
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 0.84±0.03 4.10±0.95 2.69±0.73 2.36±0.34
Reduction ... ... −34%* −42%
Mean±SEM (corrected) ... 3.27±0.93 1.85±0.71 1.53±0.32
Reduction ... ... −43%* −53%
P ... ... <.01* <.05*

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.

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 (y=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. The severity of the atherosclerotic area in the 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.

![Figure 5. Atherosclerotic area percentage in abdominal aorta (A) and cholesterol (Chol) deposition in thoracic aorta (B).](http://atvb.ahajournals.org/)

![Graph](http://atvb.ahajournals.org/)
including vitamin E.39 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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