

Protection by scoparone against the alterations of plasma lipoproteins, vascular morphology and vascular reactivity in hyperlipidaemic diabetic rabbit

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1 The *in vivo* pharmacological effects of scoparone (6,7-dimethoxycoumarin) in a hyperlipidaemic diabetic rabbit model were investigated.

2 Three groups of rabbits were studied: (1) normal, (2) hyperlipidaemic and diabetic-untreated and (3) hyperlipidaemic and diabetic-scoparone treated. The hyperlipidaemic diabetic rabbits were fed with 1% cholesterol and treated with alloxan, a diabetogenic agent. The plasma levels of total cholesterol, total triglyceride, very low-density lipoprotein (VLDL) cholesterol, low density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol were markedly increased as soon as the rabbit became diabetic at the second week. Scoparone-treatment ($5 \text{ mg kg}^{-1} \text{ day}^{-1}$, s.c.) significantly reduced the plasma lipid and lipoprotein cholesterol levels of the hyperlipidaemic diabetic rabbit to 73.3% of total cholesterol, 48.3% of total triglyceride, 66.0% of VLDL cholesterol, 55.7% of LDL cholesterol and 79.5% of HDL cholesterol.

3 Six weeks after cholesterol-feeding, the aortic arch and thoracic aorta were dissected for morphological and functional studies. In vascular rings from the untreated hyperlipidaemic diabetic rabbit, there was intimal thickening with accumulation of fatty streaks, foam cells and migration of smooth muscle cells to the intima. In the rabbits treated with scoparone, there were fewer pathological morphology changes found in vascular segments than in the untreated hyperlipidaemic diabetic rabbits.

4 In the vascular reactivity experiments, the phenylephrine-induced contraction and nitroprusside-induced dilatation did not differ significantly among the three rabbit groups, except that the contraction was enhanced in the thoracic aorta of hyperlipidaemic diabetic rabbits either untreated or treated with scoparone, as compared to the normal group, and the sensitivity to nitroprusside was increased in the thoracic aorta of the scoparone-treated group as compared to the untreated group.

5 The endothelium-dependent dilatation induced by acetylcholine was significantly attenuated in both the aortic arch and thoracic aorta from the hyperlipidaemic diabetic rabbits as compared to the normal rabbits. This attenuation was partially prevented, when scoparone (5 mg kg^{-1}) was administered daily.

6 These results suggest that scoparone protects against some alterations of plasma lipoproteins, vascular morphology and vascular reactivity in the hyperlipidaemic diabetic rabbit. These protective effects of scoparone may be partly related to its free radical scavenging property.

Keywords: Scoparone; coumarin derivative; hyperlipidaemic diabetic rabbit; hypolipidaemic effect; antiatherogenic effect; vascular reactivity

Introduction

Scoparone (6,7-dimethoxycoumarin), an active principle from the hypolipidaemic Chinese herb *Artemisia scoparia*, has been found to possess free-radical scavenging properties (Huang *et al.*, 1992b) and to exhibit vasodilator and antiproliferative effects (Huang *et al.*, 1991a; 1992a,b; 1993). In this study, the *in vivo* pharmacological effects of scoparone in the hyperlipidaemic diabetic rabbit were investigated. Hyperlipidemia is often associated with diabetes and the abnormal lipoprotein metabolism may be one of the reasons for this (Rosenko *et al.*, 1980; Bierman, 1991). The development of drugs with a cholesterol-lowering effect on hyperlipidaemic diabetic subjects is important for preventing the vascular complications of atherosclerosis and diabetes. During the course of studying the pharmacological actions of hypolipidaemic Chinese herbs, we evaluated the hypolipidaemic effects of some crude extracts and active principles from Chinese herbs in a hyperlipidaemic diabetic rabbit model. In this model, the cholesterol-fed rabbit was treated with diabetogenic agent, alloxan, to elevate more markedly the plasma lipid and lipoprotein lipid levels at the earlier weeks

as compared to the animal fed with cholesterol only (Huang *et al.*, 1991b). The effects of scoparone on plasma lipid and lipoprotein lipid levels were investigated in this rabbit model. Moreover, as hypercholesterolaemia is known to induce important morphological and functional changes in the arterial wall, we also examined the vascular morphology and reactivity of the thoracic aorta and aortic arch from untreated and scoparone-treated hyperlipidaemic diabetic rabbits.

Methods

Animals

The effects of scoparone were evaluated by using three rabbit groups, normal, hyperlipidaemic diabetic (cholesterol-fed alloxan-diabetic) and scoparone-treated (scoparone-treated cholesterol-fed alloxan-diabetic) rabbit groups. Each group contained six rabbits. The three groups of rabbits were age-matched and weight-matched. Hyperlipidaemic cholesterol-fed alloxan-diabetic rabbits were prepared in our laboratory (Huang *et al.*, 1991b). Rabbits (2.0–2.5 kg) were fed with a

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specific diet containing 1% cholesterol and 20% corn oil. In the second week, diabetes was induced by intravenous administration of a 10% solution of alloxan monohydrate freshly prepared in saline (60 mg kg^{-1}). In the scoparone-treated rabbit group, cholesterol-fed alloxan-diabetic rabbits were treated by daily injection of scoparone (5 mg kg^{-1} , s.c.) starting at the time of the administration of alloxan. Six weeks after cholesterol feeding, the aortic arch and thoracic aorta were dissected for morphological and functional studies.

Plasma lipid levels, blood pressure and blood glucose

The body weight, blood pressure, blood glucose concentration and plasma lipid, lipoprotein lipid concentrations of rabbits were measured every week. The arterial blood pressure was measured by the tail plethysmographic method (PE 300, Narco Biosystem, U.S.A.) (Huang, 1984). Before the start of experiments, rabbits (restrained, at room temperature, with 7/16 inch i.d. cuff) were trained to enable reproducible control values to be obtained. The blood glucose concentration was measured with a glucose analyser (YSI model 23A, Scientific Division, U.S.A.). Lipoprotein fractions were isolated by repeated ultracentrifugations according to the method of Buege & Aust (1978). Plasma was adjusted to the desired density by the addition of NaBr. Lipoprotein fractions including very low-density lipoprotein (VLDL; $d < 1.019 \text{ g ml}^{-1}$), low-density lipoprotein (LDL; $d = 1.019\text{--}1.063 \text{ g ml}^{-1}$) and high-density lipoprotein (HDL; $d = 1.063\text{--}1.21 \text{ g ml}^{-1}$) were obtained sequentially by differential ultracentrifugation ($110,000 g$ at 10°C). The plasma total cholesterol, total triglyceride and lipoprotein cholesterol levels were measured by Merck assay kits (Darmstadt, Germany).

Vascular morphology

Vascular segments were fixed in 10% formalin. The fixed tissue samples were dehydrated and embedded in paraffin, cut into $5 \mu\text{m}$ sections and stained with Movat's pentachrome stain. Histological sections were projected, and the intima/media index (intimal/media area times 100) was determined by computerized planimetry.

Vascular reactivity

The aortic arch and thoracic aorta were excised. The vessel was dissected free from connective tissue and ring segments (3 to 5 mm) were prepared. The vascular ring was mounted between hooks and placed in a 5 ml organ bath containing Krebs solution at 37°C and bubbled with 5% CO_2 in O_2 . Isometric tension was measured with a transducer (Harvard Bioscience, South Natick, MA, U.S.A.) attached to tissue bath computer (Buxco electronics, Sharon, CT, U.S.A.). Rings were stretched to the optimal length for isometric contraction (Huang & Lee, 1985). The preparations were then allowed to equilibrate for 120 min.

Vascular responses to vasoconstrictor (phenylephrine) and vasodilators (acetylcholine and nitroprusside) were studied. Concentration-response curves of the vasoconstrictor were obtained by cumulative additions of phenylephrine. In order to demonstrate the effect of the vasodilators, a dose of phenylephrine ($3 \times 10^{-6}\text{--}10^{-5} \text{ M}$) producing a sustained contraction was used to precontract the vascular ring. After the addition of phenylephrine (when a stable plateau was reached) cumulative doses of vasodilator were added. The contractions elicited by phenylephrine was expressed as the gram tension increase. The dilatations elicited by acetylcholine and nitroprusside were expressed as percentages of the maximal tension increase produced by phenylephrine. The concentration evoking 50% of the maximal response (EC_{50} value) for each experiment was estimated graphically from the dose-response curve.

Materials

Scoparone was purchased from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). (-)-Phenylephrine HCl, acetylcholine chloride, sodium nitroprusside, cholesterol, and sodium bromide were purchased from Sigma Co. (St. Louis, MO, U.S.A.). New Zealand rabbits were supplied from the animals centre of National Taiwan University, Taipei, Taiwan.

Statistics

The data were expressed as means \pm s.e.mean. For statistical analysis, Student's *t* test for unpaired observation was used. *P* values less than 0.05 were considered to be significant.

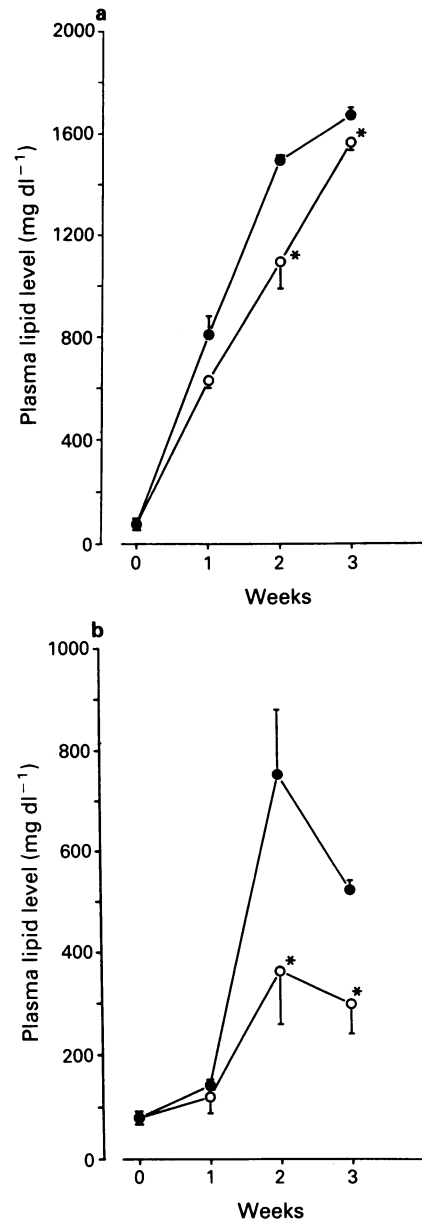


Figure 1 Effects of scoparone on plasma (a) total cholesterol and (b) total triglyceride concentrations in hyperlipidaemic diabetic rabbits. The plasma levels of total cholesterol and total triglyceride in hyperlipidaemic diabetic (●) and scoparone-treated (○) groups were measured. Hyperlipidaemic diabetic rabbits were fed with a specific diet containing 1% cholesterol and 20% corn oil and diabetes was then induced by 10% alloxan solution (60 mg kg^{-1}) at the second week. The scoparone-treated hyperlipidaemic diabetic group was treated by daily injection of scoparone (5 mg kg^{-1} , s.c.) after the administration of alloxan. Points represent the means \pm s.e.mean ($n = 6$). * $P < 0.05$, compared to the hyperlipidaemic diabetic group.

Table 1 Effect of scoparone on plasma lipid and lipoprotein lipid concentrations in the hyperlipidaemic diabetic rabbit

	n	Total cholesterol (mg dl ⁻¹)	Total triglyceride (mg dl ⁻¹)	Lipoprotein cholesterol (mg dl ⁻¹)		
				VLDL	LDL	HDL
Normal group	6	76.0 ± 2.2	80.0 ± 7.5	29.7 ± 2.0	21.8 ± 2.5	33.0 ± 5.2
Hyperlipidaemic diabetic group	6	1492.7 ± 6.0 ^a	752.8 ± 128.3 ^a	1182.3 ± 24.6 ^a	219.1 ± 30.6 ^a	58.0 ± 1.3 ^a
Scoparone-treated group	6	1093.8 ± 105.1 ^b (73.3%)	363.3 ± 103.5 ^b (48.3%)	778.8 ± 20.8 ^b (66.0%)	122.1 ± 27.5 ^b (55.7%)	46.1 ± 0.7 ^b (79.5%)

The values are means ± s.e.mean of plasma lipid and lipoprotein lipid concentrations at the end of the second week. The values in parentheses express percentage of values in the hyperlipidaemic diabetic group. The hyperlipidaemic diabetic group was fed with a specific diet containing 1% cholesterol and 20% corn oil and diabetes was then induced by bolus intravenous injection of 10% alloxan solution (60 mg kg⁻¹) at the second week. The scoparone-treated group was fed with the specific diet, injected with alloxan and treated by daily injection of scoparone (5 mg kg⁻¹ day⁻¹, s.c.) starting at the time of the administration of alloxan.

^aP < 0.05, compared to normal group; ^bP < 0.05, compared to hyperlipidaemic diabetic group.

Table 2 Responsiveness of vascular rings from normal, untreated and scoparone-treated hyperlipidaemic diabetic rabbits to vasoactive agents

Vessel	n	Phenylephrine		Acetylcholine		Nitroprusside	
		EC ₅₀ (10 ⁻⁷ M)	T _{max} (g)	EC ₅₀ (10 ⁻⁸ M)	Max. response (%)	EC ₅₀ (10 ⁻⁷ M)	Max. response (%)
Normal group							
Aortic arch	6	5.8 ± 0.5	9.2 ± 0.6	5.5 ± 1.7	50.0 ± 2.8	14.8 ± 5.3	85.8 ± 3.7
Thoracic aorta	12	3.7 ± 0.6	5.8 ± 0.2	6.9 ± 0.7	77.5 ± 1.8	8.3 ± 2.1	96.5 ± 0.9
Hyperlipidaemic diabetic group							
Aortic arch	5	6.5 ± 1.4	7.5 ± 1.4	55.0 ± 20.0 ^a	35.6 ± 1.2 ^a	11.8 ± 4.4	85.0 ± 3.3
Thoracic aorta	12	2.5 ± 0.8	6.8 ± 0.3 ^a	11.3 ± 0.3 ^a	59.7 ± 2.6 ^a	5.9 ± 0.5	93.2 ± 1.8
Scoparone-treated group							
Aortic arch	5	6.3 ± 1.7	8.0 ± 0.5	5.7 ± 0.6 ^b	37.5 ± 3.3 ^a	5.0 ± 1.1	88.8 ± 0.3
Thoracic aorta	12	2.8 ± 0.5	6.8 ± 0.3 ^a	7.7 ± 0.7 ^b	72.1 ± 4.0 ^b	3.1 ± 0.7 ^b	95.0 ± 1.0

The values are means ± s.e.mean. The hyperlipidaemic diabetic group was fed with a specific diet containing 1% cholesterol and 20% corn oil and diabetes was then induced by intravenous injection of 10% alloxan solution (60 mg kg⁻¹) at the second week. The scoparone-treated group was fed with the specific diet, injected with alloxan and treated by daily injection of scoparone (5 mg kg⁻¹ day⁻¹, s.c.) after the administration of alloxan. Six weeks after cholesterol-feeding vessels were dissected out from three rabbit groups for vascular reactivity experiments.

^aP < 0.05, compared to normal group. ^bP < 0.05, compared to hyperlipidaemic diabetic group.

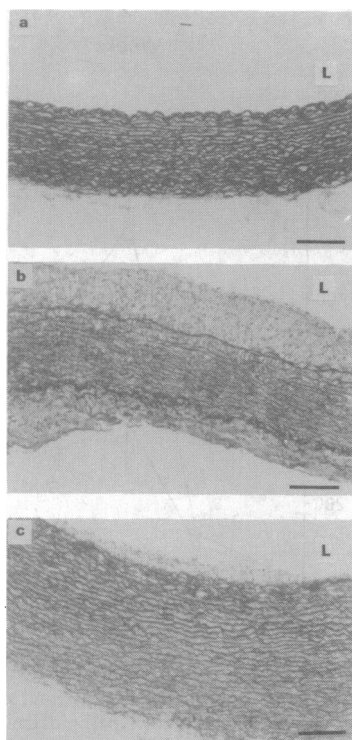


Figure 2 Light microscopical features of cross sections of the aortic arches obtained from (a) normal, (b) hyperlipidaemic diabetic and (c) scoparone-treated hyperlipidaemic diabetic rabbit groups. Movat's pentachrome stain, 150 ×. Bar = 70 μm. Marked intimal thickening, significant fibrous plaque and foam cells were found in vessels from the hyperlipidaemic diabetic group. In vessels from the scoparone-treated group, less intimal thickening, no significant fibrous plaque and fewer foam cells were observed. L: lumen.

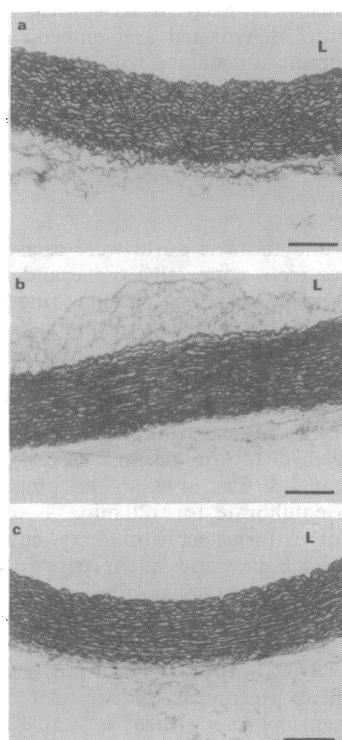


Figure 3 Light microscopical features of cross sections of the thoracic aortae obtained from (a) normal, (b) hyperlipidaemic diabetic and (c) scoparone-treated hyperlipidaemic diabetic rabbit groups. Movat's pentachrome stain, 150 ×. Bar = 70 μm. Marked intimal thickening, significant fibrous plaque and foam cells were found in vessels from the untreated hyperlipidaemic diabetic group. In vessels from the scoparone-treated group, less intimal thickening, less fibrous plaque and fewer foam cells were observed. L: lumen.

Results

Baseline data

During treatment in rabbits, scoparone ($5 \text{ mg kg}^{-1} \text{ day}^{-1}$) was well tolerated and no serious side effect was observed. Although the body weight increased with time during the experimental period (2.3 ± 0.1 to $2.5 \pm 0.1 \text{ kg}$), there was no significant difference in body weight between the scoparone-treated and the untreated hyperlipidaemic diabetic rabbits (2.3 ± 0.1 to 2.5 ± 0.1 vs. 2.3 ± 0.1 to $2.6 \pm 0.1 \text{ kg}$). There was no significant difference in the blood pressure between scoparone-treated and untreated rabbits ($98.9 \pm 5.0 \text{ mmHg}$ for basal control values; 107.6 ± 4.6 vs. $118.6 \pm 7.2 \text{ mmHg}$ at the 6th week). The mean values of arterial blood pressure of the scoparone-treated group were lower than those of the untreated hyperlipidaemic diabetic group after the 3rd week (105.2 ± 5.7 vs. $118.9 \pm 5.9 \text{ mmHg}$ at the 4th week). However, the differences in blood pressure between treated and untreated groups were inconsistent and did not achieve significance during the 6-week period. Upon administration of alloxan in cholesterol-fed rabbit at the second week, the blood glucose concentration was elevated to a level above 250 mg dl^{-1} within 48 h. The blood glucose concentration of untreated hyperlipidaemic diabetic rabbit was elevated from 141.2 ± 3.5 to $262.0 \pm 46.6 \text{ mg dl}^{-1}$. Scoparone-treatment reduced the blood glucose to $223.5 \pm 28.0 \text{ mg dl}^{-1}$. However, the differences in blood glucose concentrations between treated and untreated groups did not achieve significance.

In the normal group, the plasma total cholesterol, total triglyceride and lipoprotein cholesterol concentrations did not change significantly during the 6-week period. In the untreated cholesterol-fed alloxan-diabetic group, the total plasma cholesterol and triglyceride levels were markedly increased as soon as the rabbit became diabetic at the second week. The increases in the plasma cholesterol and triglyceride in cholesterol-fed alloxan-diabetic rabbits were more marked than those in rabbits fed with cholesterol only (Huang *et al.*, 1991b). The plasma total cholesterol concentrations were greatly increased at the second week ($1492.7 \pm 6.0 \text{ mg dl}^{-1}$) (Figure 1a) and continued to rise throughout the study ($2123.8 \pm 266.8 \text{ mg dl}^{-1}$ at the 6th week). The plasma total triglyceride had a peak value at the second week ($752.8 \pm 128.3 \text{ mg dl}^{-1}$) and decreased at the third week (Figure 1b), then remained stable ($523.0 \pm 19.1 \text{ mg dl}^{-1}$ at the 6th week). Table 1 shows the lipid profiles of three rabbit groups at the end of the second week. Scoparone-treatment ($5 \text{ mg kg}^{-1} \text{ day}^{-1}$) significantly reduced the plasma lipid and lipoprotein cholesterol concentrations of hyperlipidaemic diabetic rabbits at the second week to 73.3% of total cholesterol, 48.3% of total triglyceride, 66.0% of VLDL cholesterol, 55.7% of LDL cholesterol and 79.5% of HDL cholesterol. At the 6th week, scoparone-treatment still significantly reduced the plasma total cholesterol to 80%, but did not affect the plasma total triglyceride.

Vascular morphology

Six weeks after cholesterol feeding, the aortic arches and thoracic aortae were dissected out from the three rabbit groups. Microscopic evaluations of segments of aortic arch and thoracic aorta are shown in Figures 2 and 3. No lesion was noted in the blood vessels from normal group (I/M index = 0, $n = 6$, for both aortic arch and thoracic aorta) (Figures 2a and 3a). In contrast, there was intimal thickening with accumulation of fatty streaks, foam cells and migration of smooth muscle cells to the intima in vascular segments from the untreated hyperlipidaemic diabetic group (I/M index = 50 ± 3 , $n = 5$, and 41 ± 7 , $n = 6$, for aortic arch and thoracic aorta, respectively) (Figures 2b and 3b). Of the arteries obtained from the hyperlipidaemic diabetic rabbits, the aortic arch was more affected by the lesion than the thoracic aorta. In the rabbits treated with scoparone, there

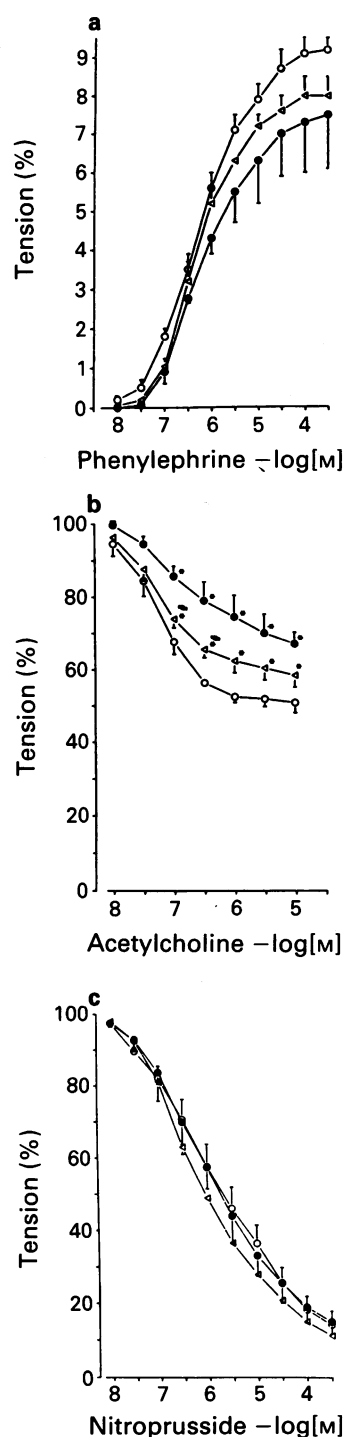


Figure 4 Concentration-response curves to (a) phenylephrine, (b) acetylcholine and (c) nitroprusside in aortic arch rings obtained from normal, hyperlipidaemic diabetic and scoparone-treated hyperlipidaemic diabetic rabbit groups. Contractile or dilator responses were recorded in vascular rings from normal (○), untreated hyperlipidaemic diabetic (●) and scoparone-treated hyperlipidaemic diabetic (△) groups. Contractile response was expressed in grams (Tension, g). Dilator response was expressed as percentage of the maximum tension increase produced by phenylephrine (Tension, %). Points represent the means \pm s.e.mean ($n \geq 5$). * $P < 0.05$, compared to the normal group.

was a significantly less fatty lesion found in both vessels (I/M index = 7 ± 1 , $n = 5$, and 3 ± 0.2 , $n = 6$, for aortic arch and thoracic aorta, respectively) as compared to the untreated hyperlipidaemic diabetic rabbits (Figures 2c and 3c). Less protective effect of scoparone-treatment was observed in the aortic arch than in the thoracic aorta.

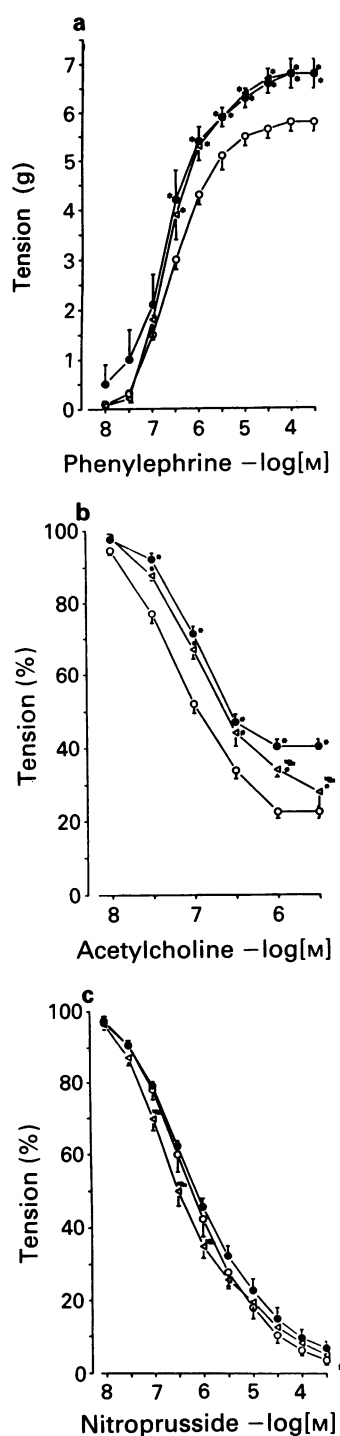


Figure 5 Concentration-response curves to (a) phenylephrine, (b) acetylcholine and (c) nitroprusside in thoracic aortic rings obtained from normal, hyperlipidaemic diabetic and scoparone-treated hyperlipidaemic diabetic rabbit groups. Contractile or dilator responses were recorded in vascular rings from normal (O), untreated hyperlipidaemic diabetic (●) and scoparone-treated hyperlipidaemic diabetic (Δ) groups. Contractile response was expressed in grams (Tension, g). Dilator response was expressed as a percentage of the maximum tension increase produced by phenylephrine (Tension, %). Points represent the means \pm s.e. mean ($n = 12$). * $P < 0.05$, compared to normal group. † $P < 0.05$, compared to untreated hyperlipidaemic diabetic group.

Vascular reactivity

Increasing concentrations of phenylephrine (10^{-8} – 10^{-4} M) evoked concentration-dependent contractions in vascular rings of the aortic arch and thoracic aorta from three rabbit groups (Figure 4a and 5a). The EC_{50} and maximal tension

(T_{max}) values of responses to phenylephrine are listed in Table 2. The EC_{50} and T_{max} values in aortic arch were not significantly changed between the hyperlipidaemic diabetic and the scoparone-treated rabbit groups. In the thoracic aorta, the contraction responses to phenylephrine were significantly enhanced in hyperlipidaemic diabetic rabbits either untreated or treated with scoparone (Figure 5a and Table 2).

When acetylcholine was added to vascular rings precontracted with phenylephrine, concentration-dependent dilations were obtained in the aortic arch and thoracic aorta at the concentration ranges of 10^{-8} – 10^{-5} M and 10^{-8} – 3×10^{-6} M, respectively (Figures 4b and 5b). In the aortic arch and thoracic aorta, the dilator responses to acetylcholine were significantly attenuated at almost all concentrations in the untreated hyperlipidaemic diabetic group as compared to the normal group. The attenuations were partially prevented when scoparone was administered daily (Figures 4b and 5b). As shown in Table 2, the sensitivity and maximal dilatation in the aortic arch and thoracic aorta were significantly reduced in the hyperlipidaemic diabetic group and scoparone-treatment significantly restored the sensitivity in both vessels, but restored only the maximal dilatation in the thoracic aorta. A dose-dependency for the scoparone-induced protective effect was observed at the dose range of 1–10 mg kg^{-1} day $^{-1}$. At 5 and 10 mg kg^{-1} day $^{-1}$, scoparone exhibited the maximum protective activity against impaired endothelium-dependent relaxation.

Nitroprusside induced a concentration-dependent dilatation in vascular rings of the aortic arch and thoracic aorta at the concentration range of 10^{-8} – 3×10^{-4} M in three rabbit groups (Figures 4c and 5c). The EC_{50} and maximal response values of nitroprusside-induced dilations did not differ significantly among the three rabbit groups (Table 2), except that the EC_{50} was decreased in the thoracic aorta of scoparone-treated group as compared to the untreated group.

Discussion

We have evaluated the *in vivo* effects of scoparone in a hyperlipidaemic diabetic rabbit model. The results demonstrate that scoparone not only reduced the elevation of plasma lipid and lipoprotein lipid levels, but also protected against some pathological alterations of vascular morphology and reactivity in the aortic arch and thoracic aorta. The hyperlipidaemic cholesterol-fed alloxan-diabetic rabbit, provides a useful model to examine the effects of compounds on lipid profile, atherogenic lesion and vascular reactivity in the hyperlipidaemic diabetic animal. Upon alloxan administration in the cholesterol-fed rabbit, the concentrations of plasma total lipid and lipoprotein cholesterol were markedly increased as soon as the rabbit became diabetic at the second week. Therefore, the hypolipidaemic effect of drugs can be easily evaluated at the earlier weeks in this animal model as compared to the animal fed with cholesterol only. By using this model we have evaluated the hypolipidaemic effects of some crude extracts and active principles from Chinese herbs. In this study, scoparone-treatment significantly reduced the elevated levels of total cholesterol, total triglyceride, very low-density lipoprotein (VLDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol in hyperlipidaemic diabetic rabbits. The reduction in HDL cholesterol was less than the other fractions. Six weeks after cholesterol feeding, the significant morphological and functional alterations were observed in vascular tissues. These pathological vascular changes may be induced by hyperlipidaemia or diabetes. Administration of scoparone to this rabbit model has been found to be associated with reduction of plasma lipids, the regression of atheroma and protection of vascular reactivity. The protective effects of scoparone on the vascular morphological and functional changes in the hyperlipidaemic diabetic rabbit were

more prominent in the thoracic aorta than in the aortic arch. Probuco, an effective antioxidant, has been reported to lower LDL and HDL levels, enhance the fractional catabolic rate of LDL, lower plasma LDL levels, enhance various components of the reverse cholesterol transport system through modifications to HDL and apo E synthesis, and prevent the oxidative modification of LDL (Schwartz, 1988). Scoparone possessing a free radical scavenging property (Huang *et al.*, 1992b) may also affect the lipoprotein levels and atherosclerotic changes as does probuco.

In our vascular activity experiments, the enhancement of phenylephrine-induced contraction was observed in the thoracic aorta from hyperlipidaemic diabetic rabbits and scoparone treatment had no effect on responses to phenylephrine. It has been reported that the contractions caused by the selective α_1 -adrenoceptor agonist, phenylephrine, are not altered in arteries obtained from hypercholesterolaemic rabbits (Henry & Yokoyama, 1980; Yokoyama *et al.*, 1983), indicating that the diabetic state of the animal may be the important factor for the enhancement of phenylephrine-induced contraction in the present study. Similarly, an increased vascular reactivity to α -adrenoceptor agonists has been reported in aorta and carotid artery from alloxan-diabetic rabbits (Cseuz *et al.*, 1973; Agrawal *et al.*, 1985). It has been demonstrated that there are α_2 -adrenoceptors but not α_1 , on endothelial cells that mediate the release of endothelium-derived relaxing factor (EDRF) (Angus *et al.*, 1986). The enhancement of phenylephrine-induced contraction may be less related to endothelial dysfunction. It has been indicated that the difference between induced contractions of healthy and diabetic vessels became evident only after denudation of endothelium and injury of endothelial cells may also contribute to the hyper-reactivity of diabetic arteries (Gebremedhin *et al.*, 1987). Several changes, such as impairment of adrenergic nerve fibres, change in calcium homeostasis and increased activity of calcium channels have also been proposed as responsible for the altered contractile responsiveness of diabetic vascular smooth muscle to α -adrenergic stimulation (Gebremedhin *et al.*, 1987). Further studies are needed to elucidate the mechanisms of the enhancement of phenylephrine-induced contraction in our

animal model. On the other hand, the endothelium-dependent dilatation to acetylcholine in rabbit aorta has been reported to be impaired by cholesterol feeding (Jayakody *et al.*, 1985). In the present study, the endothelium-dependent dilatations induced by acetylcholine were significantly attenuated in both aortic arch and thoracic aorta from the hyperlipidaemic diabetic rabbit. The impairment of the acetylcholine-induced dilatation was partially restored by daily treatment with scoparone. Components related to atherogenesis, such as endothelial cells and inflammatory cells, can produce superoxide anion, and this anion modified LDL at the extracellular space (Morel *et al.*, 1984; Parthasarathy *et al.*, 1986) and inactivates the endothelium-derived relaxing factor (EDRF) (Tagawa *et al.*, 1991). Oxidized LDL may lead to an acceleration of atherosclerotic lesion. The oxidative modification of EDRF has been reported to be a possible mechanism for the impairment of endothelium-dependent dilatation to acetylcholine in the cholesterol-fed rabbit (Mügge *et al.*, 1991). Since scoparone has been found by us to exhibit free radical scavenging activity in the vascular ring (Huang *et al.*, 1992b), it may protect against the atherosclerotic lesion and the impairment of vascular dilatation through reducing the oxidative modification of LDL and EDRF in the hyperlipidaemic diabetic rabbit. There was a decrease in the EC_{50} of nitroprusside-induced dilatation in the thoracic aorta of the scoparone-treated group as compared to the untreated group, suggesting that the endothelium-independent dilatation in the aorta may also be affected. This effect may be partly mediated through the direct activation of guanylate cyclase by scoparone (Huang *et al.*, 1992b).

In summary, scoparone-treatment reduced the elevation of plasma lipid and lipoprotein cholesterol concentrations and protected against some *in vivo* vascular morphological and functional alterations in hyperlipidaemic cholesterol-fed alloxan-diabetic rabbit. These protective effects of scoparone may be partly related to its free radical scavenging property.

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References

- AGRAWAL, D.K., BHIMJI, S. & MCNEILL, J.H. (1985). Increased vascular reactivity to agonists and prostaglandins in alloxan-treated diabetic rabbits. *Proc. Can. Fed. Biol. Soc.*, **28**, PA91.
- ANGUS, J.A., COCKS, T.M. & SATOH, K. (1986). The α adrenoceptors on endothelial cells. *Fed. Proc.*, **45**, 2355–2359.
- BIERMAN, E.L. (1991). Atherosclerosis and other forms of arteriosclerosis. In *Harrison's Principles of Internal Medicine*. 12th ed., eds. Wilson, J.D., Braunwald, E., Isselbacher, K.J., Petersdorf, R.G., Martin, J.B., Fauci, A.S. & Root, R.K. pp. 992–1001. New York: McGraw-Hill Inc.
- BUEGE, J.A. & AUST, S.D. (1978). Microsomal lipid peroxidation. *Methods Enzymol.*, **52**, 302–310.
- CSEUZ, R., WENGER, T.L., KUNOS, G. & SZENTIVANYI, M. (1973). Changes in adrenergic reaction pattern in experimental diabetes mellitus. *Endocrinology*, **93**, 752–755.
- GEbremedhin, D., KOLTAL, M.Z., POGATSA, G., MAGYAR, K. & HADHAZY, P. (1987). Differential contractile responsiveness of femoral arteries from healthy and diabetic dogs: role of endothelium. *Arch. Int. Pharmacodyn.*, **288**, 100–108.
- HENRY, P.D. & YOKOYAMA, M. (1980). Supersensitivity of atherosclerotic rabbit aorta to ergonovine. Mediation by a serotonergic mechanism. *J. Clin. Invest.*, **66**, 306–313.
- HUANG, H.C. (1984). Effects of phospholipases A_2 from *Vipera russelli* snake venom on blood pressure, plasma prostacyclin level and renin activity in rats. *Toxicol.*, **22**, 253–264.
- HUANG, H.C., CHU, S.H. & LEE CHAO, P.D. (1991a). Vasorelaxants from Chinese herbs, emodin and scoparone, possess immunosuppressive properties. *Eur. J. Pharmacol.*, **198**, 211–213.
- HUANG, H.C., HUANG, Y.L., CHANG, J.H., CHEN, C.C. & LEE, Y.T. (1992a). Possible mechanism of immunosuppressive effect of scoparone (6, 7-dimethoxy coumarin). *Eur. J. Pharmacol.*, **217**, 143–148.
- HUANG, H.C., LAI, M.W., WANG, H.R., CHUNG, Y.L., HSIEH, L.M. & CHEN, C.C. (1993). Antiproliferative effect of esculetin on vascular smooth muscle cells: possible roles of signal transduction pathways. *Eur. J. Pharmacol.*, **237**, 39–44.
- HUANG, H.C. & LEE, C.Y. (1985). Relaxant effect of phospholipase A_2 from *Vipera russelli* snake venom on rat aorta. *Eur. J. Pharmacol.*, **118**, 139–146.
- HUANG, H.C., LEE, C.R., WENG, Y.I., LEE, M.C. & LEE, Y.T. (1992b). Vasodilator effect of scoparone (6,7-dimethoxycoumarin) from a Chinese herb. *Eur. J. Pharmacol.*, **218**, 123–128.
- HUANG, H.C., WENG, Y.I., LEE, C.R., JAN, T.R. & LEE CHAO, P.D. (1991b). Mechanisms of antiatherogenic action of *Polygonum multiflorum*. *The Sixth Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, p. 207.
- JAYAKODY, R.L., SENARATNE, M.P., THOMSON, A.B. & KAPAGODA, C.T. (1985). Cholesterol feeding impairs endothelium-dependent relaxation of rabbit aorta. *Can. J. Physiol. Pharmacol.*, **63**, 1206–1209.
- MOREL, D.W., DICORLETO, P.E. & CHISOLM, G.M. (1984). Endothelial and smooth muscle cells alter low density lipoprotein *in vitro* by free radical oxidation. *Arteriosclerosis*, **4**, 357–364.

- MÜGGE, A., ELWELL, J.H., PETERSON, T.E., HOFMEYER, T.G., HEISTAD, D.D. & HARRISON, D.G. (1991). Chronic treatment with polyethylene-glycolated superoxide dismutase partially restores endothelium-dependent vascular relaxations in cholesterol-fed rabbits. *Circ. Res.*, **69**, 1293–1300.
- PARTHASARATHY, S., PRINTZ, D.J., BOYD, D., JOY, L. & STEINBERG, D. (1986). Macrophage oxidation of low density lipoprotein generates a modified form recognized by the scavenger receptor. *Arteriosclerosis*, **6**, 505–510.
- ROSENKO, J.M., BRESLOW, J.L., MIETTINEN, O.S. & GABBAY, K.H. (1980). Hyperglycemia and plasma lipid levels. A prospective study of young insulin-dependent diabetic patients. *N. Engl. J. Med.*, **302**, 650–654.
- SCHWARTZ, C.J. (1988). The probucol experience: a review of the past and a look at the future. *Am. J. Cardiol.*, **62**, 1B–5B.
- TAGAWA, H., TOMOIKE, H. & NAKAMURA, M. (1991). Putative mechanisms of the impairment of endothelium-dependent relaxation of the aorta with atheromatous plaque in heritable hyperlipidemic rabbits. *Circ. Res.*, **68**, 330–337.
- YOKOYAMA, M., AKITA, H., MIZUTANI, T., FUKUZAKI, H. & WATANABE, Y. (1983). Hyperreactivity of coronary arterial smooth muscles in response to ergonovine from rabbits with hereditary hyperlipidemia. *Circ. Res.*, **53**, 63–71.

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