The Effect of Scoparone, a Coumarin Derivative Isolated from the Chinese Crude Drug Artemisiae Capillaris Flos, on the Heart

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In the present study, scoparone isolated from Artemisia Capillaris Flos has been investigated to determine its pharmacological properties on the heart. Scoparone was found to cause the increase in coronary flow and heart rate, but did not affect cardiac output, left ventricular pressure or left ventricular work in the isolated perfused heart. Scoparone at 25 mg/kg and 50 mg/kg, *p.o.* had a marked inhibitory effect on the ST wave depression. Consequently it is suggested that scoparone has antianginal action.

Keywords *Artemisia capillaris*; Capillaris Flos; scoparone, working heart perfusion; antianginal action; vasopressin; ST wave depression

Capillaris Flos is the bud of *Artemisia capillaris* THUNB. (Compositae). As a Chinese crude drug, it has been used since antiquity for cholagogic, antipyretic, antiinflammatory and diuretic purposes in jaundice, inflammation of the liver and cholecystitis. In the course of screening test to find vasodilating drugs from among natural products, the extracts of herbal medicines were examined for their inhibitory effect on norepinephrine-induced contraction. The effect of acetone extract of *Artemisia capillaris* and scoparone (6,7-dimethoxycoumarin) on vascular smooth muscles was described in previous reports.[1,2] In the present experiments, the effects of scoparone on isolated perfused heart and on vasopressin-induced experimental angina pectoris were studied in order to examine further the action of scoparone on the cardiovascular system. First, so as to examine the effect of scoparone on cardiac functions, perfusion experiments were conducted using isolated rat heart.

Experimental

Analysis Using Working Heart Perfusion Model[3] The working heart perfusion method was used in the experiments. Wistar male rats (Shizuoka Laboratory Animal Center) weighing 300—350 g were pretreated with heparin (500 IU/kg, i.p.). Rats were then killed by means of a blow on head, the heart was immediately excised and the heart movement was stopped in ice-cold perfusion medium. The heart was then fixed to perfusion equipment through a cannula attached to the aorta and Langendorff reversed perfusion was started. Under Langendorff perfusion, the blood was washed out, and the lung and other attached tissues were removed, and the left atria was cannulated from the pulmonary vein. After confirming that the perfusion fluid was not leaking from the left atria, working heart perfusion was started. During the working heart perfusion, a preload of approximately 14 cm H_2O was maintained on the left atria and an after load of about 90 cm H_2O was kept on the aorta. The perfusion medium was a modified Krebs Henseleit solution, kept at 37 °C and aerated with a 95% O_2, 5% CO_2 gas mixture. The composition of the solution was as follows: NaCl 118.0, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.55, $NaHCO_3$ 25.0, glucose 11.0 ethylenediaminetetraacetic acid (EDTA) 0.5 mM (pH 7.4).

Following a 40 min stabilization period, the aortic flow (AF) and the coronary flow (CF), based on the flow from the pulmonary artery, were measured by the use of an electromagentic blood flowmeter (Nihon Koden MFV 1100). The left ventricular pressure (LVP) was measured by a pressure transducer (Gould Statham P231D) attached to an injection needle inserted into the left ventricular cavity through the apex, and the heart rate was periodically measured by a tachometer (Nihon Koden). In addition, the left ventricular work (LVW) was calculated according to the formula of Kannengiesser *et al.*[4] based on the cardiac output (CO), which is the sum of AF and CF, heart rate (HR) and LVP.

$$\text{LVW (mJ/s)} = \text{pressure power} + \text{kinetic power}$$

$$\text{pressure power} = 0.002222 \times P_s \times \text{CO}$$

$$\text{kinetic power} = (1/(432 \times 10)) \times d(\text{CO})/A \times (T/T_e)$$

with P_s = Peak systolic pressure (mmHg), CO = cardiac output(ml/min), A = internal cross-sectional area of aortic cannula (cm), d = desity of perfusate (g/cm), T = cycle time (ms), T_e = ejection time (ms).

The cardiac function following stabilization (approximately 40 min) was taken as normal and the effect of the test drug was measured during a 10 min perfusion with a fixed concentration of the test drug. Scoparone was dissolved in a small amount of acetone (the solvent was confirmed to have no effect on heart perfusion).

Effects of Scoparone on Experimental Anginal Model Examination Using Vasopressin-Induced ST Wave Depression[5] A male Donryu strain rat (Nihon Rat) weighing 150—200 g was given a test drug suspended in 1% carboxymethyl cellulose (CMC-Na). Fifty minutes later, the rat was anesthetized with pentobarbital sodium (50 mg/kg, i.p.). Immediately thereafter, an electrocardiogram was recorded from lead II of the electrocardiograph (ECG II) (Nihon Koden) and this was taken as the cardiogram in the normal state. Ten minutes thereafter, that is 60 min after the oral administration of the test drug, vasopressin (0.5 IU/kg) (Sigma) was intravenously (i.v.) administered from the tail vein and ECG II was recorded from 0 to 6 min. Heart rate was also recorded simultaneously from a tachometer connected to ECG II. As a control, 1% CMC-Na alone was orally administered, and in the case of nitroglycerin (GTN) alone, the rat was pretreated with GTN (i.p.) for 5 min before i.v. injection.

Drugs and compounds used for the experiments were as follows: nifedipine (Sigma), pentobarbital sodium (Nembutal injectables, Dainabot), nitroglycerin (Milisrol, Sanwa Chemical), vasopressin (Sigma). All other chemicals were purchased from Wako Junyaku Kogyo (Inc).

Results

The cardiac functions after the stabilization prior to drug perfusion were as follows; AF, 26.0±0.8 min; CF, 21.0±0.5 ml/min; CO, 46.7±1.0 ml/min; LVP, 46.9±2.4 mmHg; HR, 292.1±11.0 beats/min. Taking these values as 100, the values during the 5 min perfusion in the control group (0.1% acetone) were 95.6±1.6 for CO, 101.9±1.1 for CF, 89.1±0.8 for LVP, 103.4±1.4 for HR and 85.0±1.1% for LVW. During the perfusion with scoparone at 10^{-4} M, the values were; CO, 92.5±2.1; CF, 112.7±4.1; LVP, 85.0±0.9; HR, 115.5±2.4; LVW, 77.6±2.2%. There were significant increases in the levels of CF and HR. Further, during the perfusion with 10^{-6} M nifedipine, the parameters were: CO, 63.0±10.8, CF: 136.9±4.6, LVP: 68.2±9.8, HR: 45.0±13.1, LVW: 45.2±10.6%, showing a significant increase in CF and significant decreases in CO, LVP, HR and LVW (Figs. 1—3).

Vasopressin (0.5 IU/kg, i.v.) markedly depressed the ECG II ST wave (maximal depression of 4.84±0.6 mm in 3 min). Pretreatment of the rat for 60 min with scoparone at

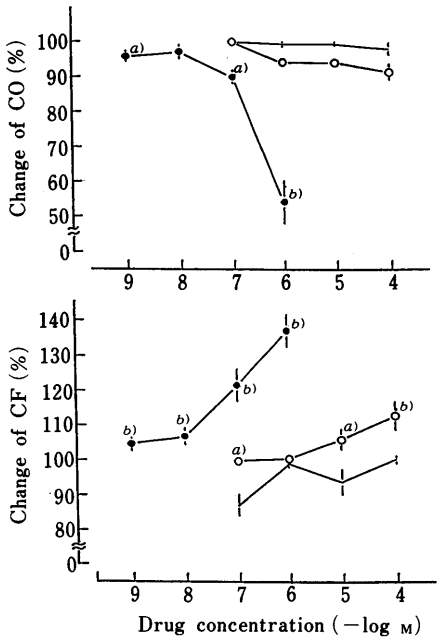

Fig. 1. Effects of Scoparone and Nifedipine on CO and CF of Perfused Working Rat Heart

$n=6$, *a*) $p<0.05$, *b*) $p<0.01$. ——, control; —○—, scoparone; —●—, nifedipine.

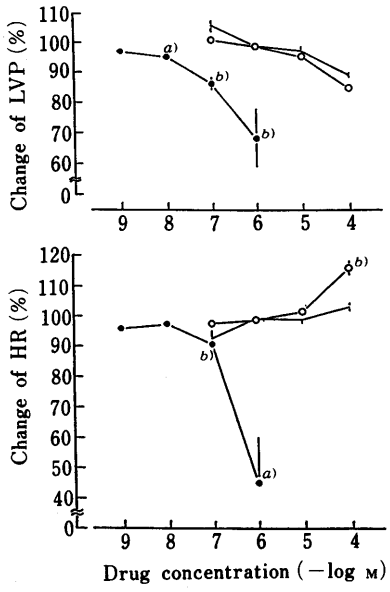

Fig. 2. Effects of Scoparone and Nifedipine on LVP and HR of Perfused Working Rat Heart

$n=6$, *a*) $p<0.05$, *b*) $p<0.01$. ——, control; —○—, scoparone; —●—, nifedipine.

25 and 50 mg/kg significantly inhibited the depression of the ECG ST wave induced by vasopressin, in a dose-dependent manner. Nifedipine, a dihydropyridine Ca^{2+}-blocker used here as a reference drug, at 1 mg/kg and GTN, a nitrous acid compound, at 3 mg/kg i.p. (5 min of pretreatment) had significant inhibitory actions. However, dipyridamole at 30 mg/kg did not show any inhibition and actually increased the ST wave depression compared with the control (Figs. 4 and 5).

Discussion

Increase in intracellular free Ca^{2+} concentration is

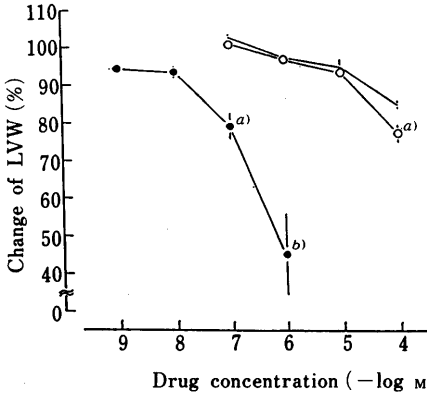

Fig. 3. Effects of Scoparone and Nifedipine on LVW of Perfused Working Rat Heart

$n=6$, *a*) $p<0.05$, *b*) $p<0.01$. ——, control; —○—, scoparone; —●—, nifedipine.

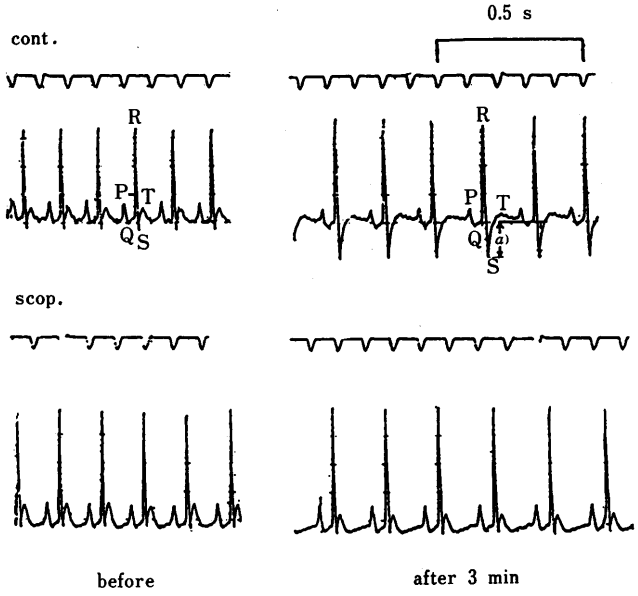

Fig. 4. Effects of Scoparone on Vasopressin Induced ST Depression in Male Donryu Strain Rat (150—200 g) Anesthetized by 50 mg/kg Pentobarbital Sodium

Drugs were orally administered 60 min before i.v. injection of 0.5 IU vasopression. cont., control (0.1% CMC-Na); scop., scoparone 50 mg/kg, *p.o.* *a*) ST shifts.

thought to be due to Ca^{2+} influx through the sarcolemmal membrane and Ca^{2+} release from intracellular Ca^{2+} stores (mainly sarcoplasmic reticulum: SR). As for Ca^{2+} influx through the sarcolemma, there are two types of channels involved in active influx, *i.e.*, -voltage-dependent Ca^{2+} channels (V.D.C.) activate by membrane depolarization and receptor-operated Ca^{2+} channels (R.O.C.) activated by activation of receptors not necessarily accompanied with depolarization.[6] Passive influx of Ca^{2+} also occurs.[6] Verapamil and nifedipine, organic Ca^{2+}-blockers, inhibit mainly Ca^{2+}-influx through V.D.C. and GTN and sodium nitroprusside (nitrocompounds) inhibit Ca^{2+}-influx through R.O.C. as well as Ca^{2+} release from SR.[7] In isolated vascular smooth muscles, scoparone has been shown to cause a parallel shift of the concentration-response curve only for NE,[1] and to inhibit extracellular Ca^{2+} influx through R.O.C. and Ca^{2+} release from Ca^{2+} stores, in a manner relatively similar to GTN.[2] In the present experi-

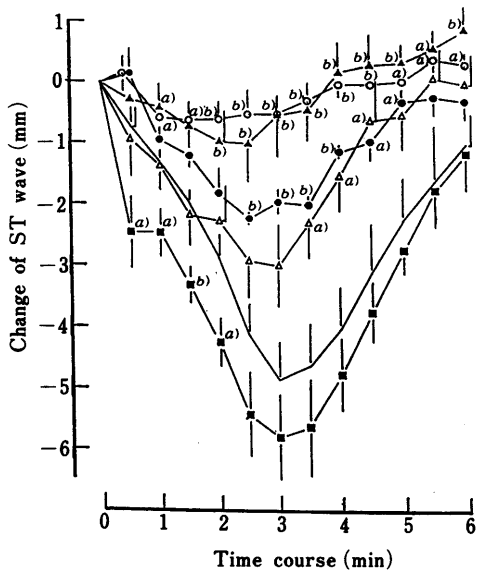

Fig. 5. Effects of Scoparone, Nifedipine, Nitroglycerin and Dipyridamole on Vasopressin Induced ST Depression in Male Donryu Strain Rats Anesthetized by 50 mg/kg Pentobarbital Sodium

n=6—7, *a*) $p<0.05$, *b*) $p<0.01$. Drugs were orally administered 60 min before i.v. administration of 0.5 IU vasopressin. ——, control; —○—, scoparone 50 mg/kg; —△—, scoparone 25 mg/kg; —▲—, nifedipine 1 mg/kg; —●—, nitroglycerin 3 mg/kg; —■—, dipyridamole 30 mg/kg.

ment using isolated rat perfused heart in order to examine the effect of scoparone on cardiac function, scoparone significantly increased CF, though the effect was weaker than that of nifedipine, a Ca^{2+}-blocker whose mechanism of action is different from that of scoparone. However, the degree of reduction in the levels of CO, LVP and LVW by scoparone was much less than that by nifedipine.

In the working heart perfusion method used in the present experiments, due to the cannulation of the pulmonary vein, the perfusion fluid is moved by the heart, working as a pump, from the left atria through the left ventricle to the aorta in the physiological sequence. Further, the approximate work applied externally by the heart can be estimated in this method, which is therefore superior to the Langendorff method of reversed perfusion from the aorta.

In the present experiments, GTN, whose mechanism of action is relatively similar to that of scoparone, was not used as the reference drug, since GTN is not only unstable in the heart but also possesses an NO_2 moiety which may bind to polyethylene tubing used in the working heart perfusion equipment. However, according to Sakamoto *et al.*,[8] GTN selectively dilates relatively large coronary vessels and therefore it only slightly increases CF in the normal heart as compared to experimental disease models. Therefore, based on the pattern and the magnitude of the vasodilating action of scoparone, the increase in CF caused by scoparone at 10^{-4} M to 112.7±4.1 (%) is due to the coronary dilatory action of scoparone. It is known that in cardiac muscles, there are no R.O.C., but only V.D.C. Nifedipine, a Ca^{2+}-blocker, inhibited CO, LVP, HR and LVW, due to inhibition of slow channels in the cardiac muscle cells. The inhibitory effect of scoparone, on CO, LVP and LVW, however, is small, since the effect of scoparone is negligible on Ca^{2+}-influx through V.D.C.

In the present experiment, the antianginal action of scoparone was examined in vasopressin-induced ST wave depression in Donryu strain rats according to the method of Hatano *et al.*[5] Vasopressin, an antidiuretic hormone, at high concentrations strongly contracts coronary vessels especially,[9] thereby causing ST wave depression of the lead II electrocardiogram in myocardial ischemia.[10] Scoparone, however, at 25 and 50 mg/kg, *p.o.*, had a marked inhibitory effect on the ST wave depression, in a dose-dependent manner. Based on the results in *in vitro* experiments, this may be due to the inhibition of coronary vasoconstriction, but it is more likely to be due to a decrease in afterload through vasodilation of resistance vessels and reduction of venous return through vasodilation of veins, both of which act synagistically to reduce cardiac work load. Even though the effect of scoparone was 50 times weaker than that of the Ca^{2+}-blocker, nifedipine, it is interesting to note that scoparone in an oral administration showed a significantly greater inhibitory action as compared to GTN, whose mechanism of action *in vitro* is relatively similar to that of scoparone. In addition, the fact that dipyridamole, developed as a coronary vasodilator, had no effect in the present experiment may be due to the so-called coronary steal syndrome,[11] in which dipyridamole mainly dilates healthy small coronary arteries leading to a decreased blood flow in the ischemic region of large coronary arteries. These results indicate that scoparone, as a vasodilater, possesses antianginal action.

References and Notes

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