Protective effects of xanthones against myocardial ischemia-reperfusion injury in rats

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KEY WORDS xanthone; *Swertia davidi* Franch; myocardial reperfusion injury; heart function tests; lipid peroxidation

ABSTRACT

AIM: To investigate the protective effect of xanthones against myocardial ischemia-reperfusion injury in rats. **METHODS:** Ischemia-reperfusion injury was induced by 20 min of global ischemia and 40 min of reperfusion in isolated rat hearts or 60-min coronary artery occlusion and 180-min reperfusion *in vivo,* respectively. Heart rate, coronary flow, left ventricular pressure (LVP), and its first derivative ($\pm dp/dt_{max}$) were recorded, and the activity of creatine kinase in coronary effluent and malondialdehyde contents in myocardial tissues were measured *in vitro*. The activity of serum creatine kinase and myocardium infarct size were measured *in vivo*. **RESULTS:** Xanthones (90 or 300 μg/L) caused a significant improvement of cardiac function (LVP and $\pm dp/dt_{max}$) and a decrease in the release of creatine kinase in coronary effluent as well as the level of malondialdehyde in myocardial tissues. Xanthones (0.5 or 1.0 mg/kg) also markedly decreased infarct size and the release of creatine kinase *in vivo*. **CONCLUSION:** Xanthones protect the myocardium against the damages induced by ischemia-reperfusion in rats, and the effect of xanthones may be related to the inhibition of lipid peroxidation.

INTRODUCTION

Swertia davidi Franch (*Gentianaceae*) is a commonly used Chinese medicinal herb. Xanthones, a main component extracted from *Swertia davidi* Franch, have extensive pharmacological actions[1]. Previous investigations have shown that some xanthones have a potent antioxidant activity and inhibit lipid peroxidation stimulated by $FeCl_2$-ADP or CCl_4-NADPH mixture in the rat liver homogenate and block the oxidation of low-density lipoprotein *in vitro* and *in vivo*[1-5]. Recently, it has been reported that some xanthones decrease the incidence of ventricular arrhythmia induced by adrenaline or ischemia-reperfusion[6,7].

Oxygen-free radical formation and intracellular Ca^{2+} overload are strongly implicated as important pathophysiological mechanisms mediating myocardial ischemia-reperfusion injury[8-10]. It has been reported that free radical scavengers such as peroxide dismutase and catalase, or some medical plants which have antioxidant properties such as mognolol or *Ginkgo biloba* prevent the myocardium against damages due to ischemia-reperfusion[11-15]. The cardioprotective effect of some Ca^{2+} channel blockers such as verapamil was related to the reduction of lipid peroxides[16]. According to antioxidant properties and the decreased intracellular Ca^{2+} level of xanthones[17,18], in the present study, we examined the protective effect of xanthones against myocardial injury induced by ischemia-reperfusion in rats.

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Received 2002-01-10 Accepted 2002-10-15

MATERIALS AND METHODS

Reagents Xanthones (Faculty of Pharmacy, Xiangya Medical College, Central South University, China), of the purity of 63.5 %, was initially dissolved in ethanol and further diluted in Krebs-Henseleit solution to proper final concentration. The final concentration of ethanol in solution did not exceed 0.1 %. Verapamil was obtained from Hefeng Pharmaceutics Co Ltd (Shanghai, China). Creatine kinase assay kits and malondialdehyde assay kits were obtained from Zhongsheng Bioengineering Co (Beijing, China) and Juli Bioengineering Co (Nanjing, China), respectively.

Preparation of the isolated heart Male Wistar rats (Laboratory Animal Center, Xiangya Medical College, Central South University, Grade II, Certificate No 20-011) weighing 200 to 250 g were anesthetized by ip administration of sodium pentobarbital (60 mg/kg). The hearts were excised and rapidly attached to a Langendorff apparatus via the aorta for retrograde perfusion with Krebs-Henseleit buffer solution (mmol/L: NaCl 119.0, $NaHCO_3$ 25.5, KCl 4.3, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, and glucose 11.0). The perfusate solution was equilibrated with 95 % O_2 and 5% CO_2, maintained at 37 °C and pH 7.4. Perfusion pressure was maintained at 80 cmH_2O.

A water-filled latex balloon connected to a pressure transducer was inserted into the left ventricle via the mitral valve. The balloon was then inflated with water to maintain a left ventricular end-diastolic pressure of 2 to 3 mmHg. Left ventricular pressure, its first derivatives ($\pm dp/dt_{max}$), and heart rate were continuously monitored. The resulting electric signals were digitized by a MacLab analogue-to-digital converter and recorded by a Power Macintosh 7220 computer. Coronary flow (CF) was measured by timed collection of coronary effluent.

Surgical preparation Male Wistar rats were anesthetized with sodium pentobarbital (60 mg/kg, ip), and then mechanically ventilated with room air using a positive pressure ventilator. The ventilation rate was maintained at 35-40 strokes per min with a tidal volume of approximately 15 mL/kg body weight. Electrocardiograph (ECG) leads were connected to the chest and limbs for continuous ECG monitoring throughout the experiment. A left thoracotomy was performed in the fourth intercostal space and the pericardium was opened to expose the heart. A 4–0 silk suture was passed around the left coronary artery and a snare was formed by passing both ends of the suture through a piece of polyethylene tubing. Occlusion of the coronary artery, by clamping the snare against the surface of the heart, caused an area of epicardial cyanosis with regional hypokinesis and ECG changes. Reperfusion was achieved by releasing the snare and was confirmed by conspicuous hyperaemic blushing of the previously ischemic myocardium and gradual resolution of the changes in the ECG signal. The sham group underwent the same procedure but without clipping of the coronary artery.

Measurement of creatine kinase activity Samples of coronary effluent after 5-min reperfusion were collected in the isolated hearts. At the end of 3-h reperfusion , blood samples were collected from the carotid artery *in vivo*. The activity of creatine kinase was assayed spectrophotometrically.

Measurement of malondialdehyde content At the end of 3-h reperfusion, the left ventricular myocardium of each heart was washed with cold salines, and then excised and added to 10 volumes of cold saline. The individual tissue samples were homogenized with tissue homogenizer and centrifugated at 2000×*g* for 15 min. The thiobarbituric acid reactive substance, reflecting levels of lipid peroxidation, in homogenate was measured by a spectrofluorometer and expressed as the amount of malondialdehyde content.

Infarct size and risk area At the end of 3-h reperfusion, the left coronary was reoccluded, and 1 mL Evans blue (1 %) was injected into the ventricular cavity to mark the original area at risk of infarction. The heart was excised, frozen, and then sliced into 1-mm thick sections from apex to base. The slices were incubated in 1 % triphenyl tetrazolium chloride phosphate buffer solution at 37 °C for 20 min to stain the viable myocardium brick red. The samples were then fixed in a 10 % formalin solution for 24 h. Sections were traced onto acetate sheets. The area of infarct and risk zone were determined by planimetry of the tracings.

Experimental protocols Seven groups of animals were designed to test the protective effect of xanthones on the myocardium *in vitro*. All hearts had an initial stabilization period for 20 min. In the control group, hearts were perfused with Krebs-Henseleit solution throughout the experiment. The ischemia-reperfusion group experienced 20-min global ischemia and 40-min reperfusion. For xanthones and verapamil, hearts were perfused with xanthones (30, 90 or 300 μg/L) or verpamil (10 μg/L) for 10 min before ischemia,

and then the drugs remained in the perfusion throughout the remainder of the experiment.

The second series of experiments were designed to further examine the protective effect of xanthones on the ischemic myocardium *in vivo*. All animals were subjected to 60 min of coronary artery occlusion followed by 180 min of reperfusion. For xanthones and verapamil, the rats were treated with xanthones (0.5 or 1.0 mg/kg, iv) or verapamil (1.0 mg/kg, iv) 5 min before ischemia.

Statistics Data were expressed as mean±SD. Statistical analysis was carried out by analysis of variance and the Newman-Keuls test. The level of significance was chosen as $P<0.05$.

RESULTS

Cardiac function and release of creatine kinase in the isolated rat heart There were no significant differences in LVP, $\pm dp/dt_{max}$, CF, and heart rate in all groups. After ischemia for 20 min, a decline in cardiac function (LVP and $\pm dp/dt_{max}$) and CF and an increase in the release of creatine kinase were shown during reperfusion. Xanthones at the concentration of 30 μg/L only caused a slight improvement of the recovery of cardiac function, while at higher doses (90 or 300 μg/L) xanthones improved the recovery of cardiac function during reperfusion. Xanthones at the concentration of 30, 90, or 300 μg/L markedly reduced the release of creatine kinase during reperfusion. The vehicle of xanthones had no effect on impairment of cardiac function and the increased release of creatine kinase during reperfusion (Tab 1, 2).

Malondialdehyde content Ischemia-reperfusion caused a significant increase in the content of malondialdehyde in myocardial tissues, that was markedly attenuated by xanthones (30, 90, or 300 μg/L) or verapamil (10 μg/L) (Tab 2).

Infarct size There were no significant differences in heart weights and risk zone among groups, indicating that the size of the risk zone was comparable in all groups. Ischemia-reperfusion caused 59 %±7 % necrosis in the area at risk. Infarct size was reduced by treatment with xanthones (0.5 or 1.0 mg/kg) or verapamil (1.0 mg/kg) (Tab 3).

Serum levels of creatine kinase Ischemia-reperfusion caused a significant increase in the serum level of creatine kinase. Similarly, the release of creatine kinase was reduced by treatment with xanthones (0.5 or 1.0 mg/kg) or verapamil (1.0 mg/kg) (Tab 3).

DISCUSSION

The present results showed that xanthones significantly improved the recovery of cardiac function and decreased the release of creatine kinase *in vitro*, and reduced infarct size and the serum level of creatine kinase during reperfusion *in vivo*. These results with previous observations that some xanthones reduced the incidence of ventricular arrhythmia during reperfusion *in vivo* and decreased the activity of lactate dehydrogenase in coronary effluent in isolated rat hearts[6,19], indicate that xanthones protect against ischemic myocardial injury.

Generation of oxygen free radicals and lipid peroxidation have been suggested to play an important role in the pathogenesis of post-ischemic myocardial dysfunction[8,9]. It has been reported that some xanthones exert antioxidative properties, and some medical plants, which have an antioxidant activity, have been shown to attenuate myocardial injury elicited by ischemia-reperfusion[1-5,12-15]. It is likely that the cardioprotective effect of xanthones is related to inhibition of peroxide generation. The present results showed that treatment with xanthones markedly enhanced the recovery of cardiac function and decreased the release of creatine kinase concomitantly with a reduction in the contents of malondialdehyde, reflecting lipid peroxidation level, in myocardial tissues.

Previous investigations have suggest that intracellular Ca^{2+} overload is an important factor in ischemia-reperfusion injury, and the occurrence of this phenomenon is related to changes of the activation of Na^+-H^+ exchange and Na^+-Ca^{2+} exchange systems in the ischemic myocardium[20]. Ca^{2+} overload increases generation of oxygen free radicals and exerts a direct toxicity to the myocardium[10]. A number of evidence has shown that Na^+-H^+ exchange inhibitors and calcium channel blockers protect the myocardium against damages due to ischemia-reperfusion[21-24]. Recently, it has been found that some xanthones inhibit the activation of Na^+-H^+ exchange system and decrease Ca^{2+} level in myocardial tissues in isolated hearts[19]. Others have shown that xanthones purified from other medical plants can block Ca^{2+} channels and inhibit Ca^{2+} influx in cardiovascular tissues[17,18]. However, the precise mechanism responsible for the cardioprotection of xanthones needs to be investigated.

Tab 1. Effects of xanthones on cardiac function. I/R: ischemia-reperfusion. n = 8. Mean±SD. $^{c}P<0.01$ *vs* control. $^{d}P>0.05$, $^{e}P<0.05$, $^{f}P<0.01$ *vs* I/R.

	Baseline	Reperfusion				
		5 min	10 min	20 min	30 min	40 min
Left ventricular pressure/mmHg						
Control	87±20	81±18	80±18	79±18	79±17	79±17
I/R	84±21	24±12^{c}	25±7^{c}	30±8^{c}	36±11^{c}	39±14^{c}
Vehicle	87±17	27±16^{d}	37±16^{d}	42±18^{d}	43±18^{d}	42±18^{d}
Xanthone 30 μg/L	84±10	32±19^{d}	43±16^{e}	50±7^{f}	51±7^{f}	52±8^{f}
Xanthone 90 μg/L	85±12	39±18^{d}	46±20^{e}	56±14^{f}	64±15^{f}	66±14^{f}
Xanthone 300 μg/L	86±12	38±16^{d}	50±17^{f}	57±16^{f}	61±13^{f}	63±13^{f}
Verapamil 10 μg/L	88±12	51±16^{f}	59±9^{f}	67±4^{f}	66±5^{f}	65±4^{f}
$+dp/dt_{max}$/mmHg·s^{-1}						
Control	2906±820	2691±652	2716±644	2685±627	2638±582	2630±591
I/R	2506±806	556±274^{c}	672±238^{c}	881±409^{c}	959±426^{c}	1074±487^{c}
Vehicle	2733±496	739±510^{d}	960±507^{d}	1276±602^{d}	1333±59^{d}	1205±622^{d}
Xanthone 30 μg/L	2562±252	816±567^{d}	1120±405^{e}	1489±323^{f}	1625±286^{f}	1700±305^{f}
Xanthone 90 μg/L	2578±343	1044±586^{d}	1280±573^{e}	1631±524^{f}	2123±432^{f}	2157±401^{f}
Xanthone 300 μg/L	2601±420	1055±413^{e}	1337±313^{f}	1637±243^{f}	1940±368^{f}	2004±378^{f}
Verapamil 10 μg/L	2832±347	1274±641^{e}	1593±434^{f}	2061±367^{f}	2026±258^{f}	2020±252^{f}
$-dp/dt_{max}$/mmHg·s^{-1}						
Control	2249±608	2092±462	2113± 448	2094±431	2060±414	2046±426
I/R	1990±526	434±218^{c}	515±143^{c}	631±162^{c}	778±260^{c}	865±358^{c}
Vehicle	2010±302	572±361^{d}	733±557^{d}	883±428^{d}	942±423^{d}	877±409^{d}
Xanthone 30 μg/L	2061±388	670±400^{d}	959±320^{e}	1229±192^{f}	1282±220^{f}	1301±264^{e}
Xanthone 90 μg/L	2116±421	791±465^{d}	973±459^{e}	1298±378^{f}	1508±379^{f}	1530±388^{f}
Xanthone 300 μg/L	2116±510	831±379^{e}	1267±375^{f}	1372±328^{f}	1438±282^{f}	1465±258^{f}
Verapamil 10 μg/L	2227±311	913±479^{e}	1156±339^{f}	1491±249^{f}	1459±230^{f}	1427±235^{f}
Coronary flow/mL·min^{-1}						
Control	14±3	13±3	13±3	13±3	13±3	13±3
I/R	12±2	8±2^{c}	8±2^{c}	8±2^{c}	8±1^{c}	8±1^{c}
Vehicle	12±2	9±2^{d}	9±2^{d}	9±2^{d}	8±2^{d}	8±2^{d}
Xanthone 30 μg/L	12±2	10±2^{d}	10±2^{d}	10±2^{d}	10±2^{d}	9±2^{d}
Xanthone 90 μg/L	12±3	10±2^{d}	10±2^{d}	10±2^{d}	10±2^{d}	10±2^{d}
Xanthone 300 μg/L	12±2	10±2^{d}	10±2^{d}	10±2^{e}	10±2^{e}	10±2^{e}
Verapamil 10 μg/L	13±2	12±2^{e}	11±2^{e}	11±3^{e}	11±3^{e}	11±3^{e}
Heart rate/min^{-1}						
Control	312±22	320±25	316±22	318±25	315±20	315±20
I/R	294±28	147±76^{c}	207±70^{c}	230±76^{c}	240±64^{c}	236±64^{c}
Vehicle	286±36	182±78^{d}	249±67^{d}	250±81^{d}	245±81^{d}	260±59^{d}
Xanthone 30 μg/L	287±26	184±69^{d}	227±25^{d}	242±25^{d}	250±28^{d}	250±28^{d}
Xanthone 90 μg/L	296±28	213±58^{d}	249±60^{d}	288±45^{d}	288±57^{d}	293±43^{d}
Xanthone 300 μg/L	284±19	195±37^{d}	222±47^{d}	241±53^{d}	241±49^{d}	240±49^{d}
Verapamil 10 μg/L	314±22	223±50^{e}	256±34^{d}	270±22^{d}	276±25^{d}	274±22^{d}

In conclusion, the present results of this study indicate that xanthones protect the myocardium against injury due to ischemia-reperfusion in rats, and that the beneficial effect of xanthones may be related to inhibi-

Tab 2. Effects of xanthones on the creatine kinase activity and malondialdehyde content in isolated rat hearts. *n*=8. Mean±SD. [c]*P*< 0.01 *vs* control. [d]*P*>0.05, [e]*P*<0.05, [f]*P*<0.01 *vs* vehicle.

	Creatine kinase/ mU·min^{-1}·g^{-1} wet weight	Malondialdehyde/ nmol·g^{-1} wet weight
Control	346±112	144± 61
I/R	1698±335[c]	284±72[c]
Vehicle	1507±186[d]	281±86[d]
Xanthone 30 μg/L	991±307[f]	200±51[e]
Xanthone 90 μg/L	728±181[f]	183±63[f]
Xanthone 300 μg/L	809±250[f]	184±38[f]
Verapamil 10 μg/L	818±236[f]	180±40[f]

tion of lipid peroxidation.

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Tab 3. Effects of xanthones on infarct size and creatine kinase activity *in vivo*. *n*=7. Mean±SD. [a]*P* > 0.05, [b]*P* < 0.05, [c]*P*<0.01 *vs* vehicle.

Group	Heart wet weight/g	Area at risk/cm^2	Infarct size/cm^2	Infarct size/ % area at risk	Creatine kinase/U·L^{-1}
Vehicle	0.75±0.03	3.1±0.5	1.8±0.4	59±7	1621±382
Xanthone 0.5 mg/kg	0.73±0.03[a]	3.1±0.5[a]	1.4±0.2[c]	46±9[b]	1081±218[c]
Xanthone 1.0 mg/kg	0.74±0.03[a]	3.1±0.3[a]	1.2±0.3[c]	40±11[c]	860±275[c]
Verapamil 1.0 mg/kg	0.73±0.03[a]	3.1±0.3[a]	1.2±0.4[c]	38±12[c]	713±159[c]
Sham	–	–	–	–	362±139[c]

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