3,4,5,6-Tetrahydroxyxanthone Protects Against Myocardial Ischemia-Reperfusion Injury in Rats

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Summary. In the present study, we tested the protective effect of 3,4,5,6-tetrahydroxyxanthone, a synthetic xanthone derivative, on myocardial ischemia-reperfusion injury in rats. Ischemia-reperfusion injury was induced by 30 min of global ischemia and 30 min of reperfusion in isolated rat hearts or 30 min coronary artery occlusion and 120 min reperfusion in vivo, respectively. Heart rate, coronary flow (CF), left ventricular pressure (LVP), and its first derivative (±dp/dtmax) were recorded, and the activity of creatine kinase in coronary effluent and tumor necrosis factor-alpha (TNF-α) content in myocardial tissues were measured in vitro. The activity of serum creatine kinase, the level of TNF-α and interleukin-6 (IL-6), and myocardial infarct size were measured in vivo. 3,4,5,6-tetrahydroxyxanthone (30, 100 or 300 µM) caused a significant improvement of cardiac function (LVP and ±dp/dtmax) and a decrease in the release of creatine kinase in coronary effluent as well as the level of TNF-α in myocardial tissues in vitro. 3,4,5,6-tetrahydroxyxanthone (0.5 or 1.0 mg/kg, i.v.) also markedly decreased infarct size and the release of creatine kinase and TNF-α, and increased serum IL-6 level and the level of TNF-α, and increased serum IL-6 level in vivo. These results suggest that 3,4,5,6-tetrahydroxyxanthone possesses a protective effect on myocardial ischemia-reperfusion injury, and that the protective effects of 3,4,5,6-tetrahydroxyxanthone may be related to inhibition of TNF-α production and stimulation of IL-6 generation by inhibition of ROS production.

Key Words. 3,4,5,6-tetrahydroxyxanthone, ischemia-reperfusion, heart, tumor necrosis factor-alpha, interleukin-6

Introduction

A great evidence has shown that reperfusion injury in the myocardium is an acute inflammatory reaction, which involves multiple cytokines. There is substantial evidence that the production of tumor necrosis factor-alpha (TNF-α) is increased during ischemia-reperfusion in the myocardium [1–4], and that anti-TNF-α treatment improves the recovery of post-ischemic myocardial function [5–7]. These findings implicate locally produced TNF-α as an important mediator of post-ischemic myocardial dysfunction, and inhibition of myocardial TNF-α production has been suggested to be a promising therapeutic strategy for the prevention of cardiac dysfunction associated with myocardial ischemia-reperfusion.

Swertia davidi Franch (Getianaceae) is a commonly used Chinese medicinal herb. Xanthone, a main component extracted from Swertia davidi Franch, has extensive pharmacological actions [8]. It has been reported that the protective effects of some xan-thones have anti-inflammatory effects [11–13]. In the present study, we examined whether the protective effect of 3,4,5,6-tetrahydroxyxanthone (Fig. 1), a synthetic xanthone, on myocardial injury induced by ischemia-reperfusion is related to inhibition of TNF-α production in rats.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing 220 to 250 g were used in this study. All animals received humane care in compliance with the “Guide for the Care and Use of Laboratory Animals” published by the National Institute of Health (NIH publication 86-23, revised 1986).

Isolated rat heart perfusion

Rats were anesthetized by intraperitoneal administration of sodium pentobarbital (60 mg/kg, i.p.). The hearts were excised and rapidly attached to a Langendorff apparatus via the aorta for retrograde perfusion with Krebs-Henseleit solution (in mM: 119.0 NaCl, 25 NaHCO3, 4.7 KCl, 1.18 KH2PO4, 1.17 MgSO4, 1.2 CaCl2, and 5.5 glucose). The perfusate solution was equilibrated with 95% O2 and 5% CO2, maintained at 37° and

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279
pH 7.4. Perfusion pressure was maintained at 80 cm H₂O.

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 (±dp/dt max) and heart rate were continuously monitored. The resulting electric signals were digitized by a Mac-Lab 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 Sprague-Dawley rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.), and then mechanically ventilated with room air using a positive pressure ventilator. The ventilation rate was maintained at 35–40 strokes min⁻¹ 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 intercostals space and the pericardium was opened to expose the heart. A 4-0 silk suture was passed around the left coronary artery, clamping the 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 hysteresis and ECG changes. Reperfusion was achieved by releasing the snare and was confirmed by conspicuous hyperaemic blushing of the previously ischemic myocardium and gradual blushing of the changes in the ECG signal. The sham group underwent the same procedure but without clamping 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 2-h reperfusion, blood samples were collected from the carotid artery in vivo and then were centrifugated at 1300 x g for 15 min (4°C). The supernatant was stored at -70°C until assay.

At the end of 2-h reperfusion, blood samples were collected from the carotid artery in vivo and then were centrifugated at 1300 x g for 15 min (4°C). The serum was stored at -70°C until assay.

The content of TNF-α in myocardial tissues or serum and the serum IL-6 content were determined by radiomunnoassay kits according to the manufacturer's protocol.

**Infarct size and risk area**

At the end of 2-h reperfusion, the left coronary was re-occluded, and 1 mL Evans blue (1%) was injected into the ventricular cavity to mark the original area at risk of infarction. The left ventricle was excised, weight, frozen, and then sliced into eight separate sections (slices) from apex to base. The slices were incubated in 1% triphenyltetrazolium chloride (TTC) 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. To calculate area of infarct and risk zone, slices were traced onto acetate sheets, each of the slices were photographed, and the area of infarction and risk zone of each slice was determined by computerized planimetry. The area of risk zone and infarct for each slice was determined by taking the basal and apical side of the infarction of each myocardial slice. Infarct size was then expressed as a percentage of the area at risk.

**Experimental protocols**

Seven groups of animals were designed to test the protective effect of 3,4,5,6-tetrahydroxanthone 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 30-min global ischemia and 30-min reperfusion. For 3,4,5,6-tetrahydroxanthones, verapamil and vehicle groups, hearts were perfused with 3,4,5,6-tetrahydroxanthone (30, 100 or 300 µM), verapamil (10 µg/L) or vehicle of xanthone (1 ml/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 was designed to further examine the protection effect of 3,4,5,6-tetrahydroxanthone on the ischemic myocardium in vivo. The sham group underwent the same procedure but without clamping of the coronary artery. The

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**Fig. 1. Chemical structure of 3,4,5,6-tetrahydroxanthone.**