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***Chrysanthemum morifolium* attenuated the reduction of contraction of isolated rat heart and cardiomyocytes induced by ischemia/reperfusion**

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The present study was aimed to investigate the effect of *Chrysanthemum morifolium* Ramat.(CM) on isolated rat heart and ventricular myocytes during ischemia/anoxia and reperfusion/reoxygenation. The ischemia/reperfusion injury was induced by ligation the left artery descending coronary of isolated rat heart for 30 min followed by 30 min reperfusion with Langendorff equipment. Cell contraction in enzymatically isolated ventricular myocytes was determined by a video tracking system. The results showed CM (0.25 g/L to 1.0 g/L) increased left ventricular developed pressure (LVDP), $\pm dp/dt_{max}$, LVDP \times HR and coronary flow (CF) and decreased heart rate (HR) in dose dependent manner. CM (0.5 g/L) attenuated the reduction of LVDP, $\pm dp/dt_{max}$ and CF caused by ischemia/reperfusion. CM (0.25 g/L to 1.0 g/L) increased peak velocity of cell shortening/relengthening ($\pm dL/dt_{max}$) and contraction amplitude (dL) of isolated ventricular myocytes in a dose-dependent way under control condition, but without significant effect on end-diastolic cell length (L0). Under anoxia 5 min followed by 10 min reoxygenation, CM attenuated the reduction in contractile parameters. The results suggest that CM processes cardioprotective effect during ischemia/anoxia and reperfusion/reoxygenation in the isolated rat heart and the ventricular myocytes.

1. Introduction

The flower of *Chrysanthemum morifolium* Ramat. (CM) has been used as a folk medicine and healthy food for many centuries in China (Zhao and Ma 1996). It has been shown that the water extract of CM increases coronary flow and contractility in isolated rabbit heart and intact dog heart (Department of Physiology of Zhejiang Medical University, 1978). Clinical trials demonstrated that CM had a beneficial effect on coronary heart disease, reducing the onset time of angina and improving the changes in the electrocardiogram (Xu and Ju 1999). However, there is lack of direct experimental data from single cardiac myocytes to demonstrate the effect of CM in anoxia and reoxygenation without the influence of nervous and humoral factors as well as the coronary arterial supply. In the present study we used the single cardiac myocyte model to determine the effects of CM on contraction parameters during anoxia and reoxygenation and used the Langendorff perfusion technique to compare the effect of CM with that in single myocyte model.

2. Investigations and results

2.1. Effect of CM on cardiac function of isolated rat heart

CM (0.25 g/L to 1.0 g/L) increased left ventricular developed pressure (LVDP), maximal rate of rise/fall of ventricular pressure ($\pm dp/dt_{max}$), LVDP \times HR, and coronary

flow (CF) of isolated rat hearts in dose dependent manner (Fig. 1A, Fig. 1B), while it reduced the heart rate (HR) at 0.5 g/L and 1.0 g/L (Fig. 1B).

2.2. Effect of CM on cardiac contractility of isolated rat heart during ischemia/reperfusion

LVDP, $\pm dp/dt_{max}$, and CF in the isolated rat hearts were reduced significantly ($P < 0.01$) after left anterior descending coronary artery (LAD) ligation for 30 min. However, treatment with CM (0.5 g/L) for 15 min prior to the ligation and during reperfusion, attenuated the reduction of the

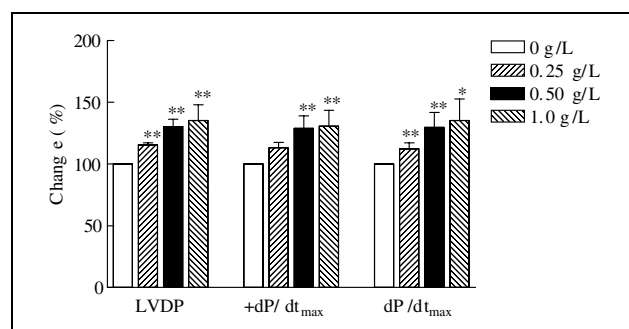


Fig. 1A: Effect of CM (0.25–1.0 g/L) on LVDP and $\pm dp/dt_{max}$ in isolated perfused rat hearts. Values are expressed as $\bar{x} \pm s$, $n = 15$. * $P < 0.05$, ** $P < 0.01$ compared with control (0 g/L)

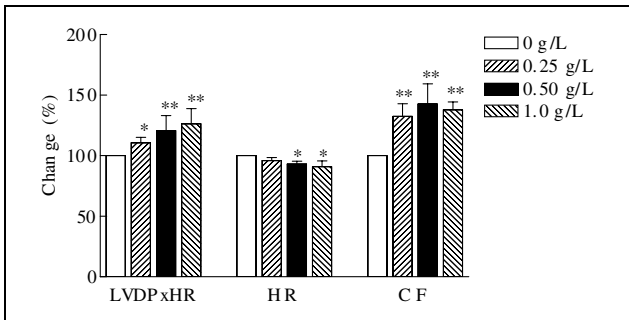


Fig. 1B: Effect of CM (0.25–1.0 g/L) on LVDP × HR, heart rate (HR) and coronary flow (CF) in isolated perfused rat hearts. Values are expressed as $\bar{x} \pm s$, n = 15. *P < 0.05, **P < 0.01 compared with control (0 g/L)

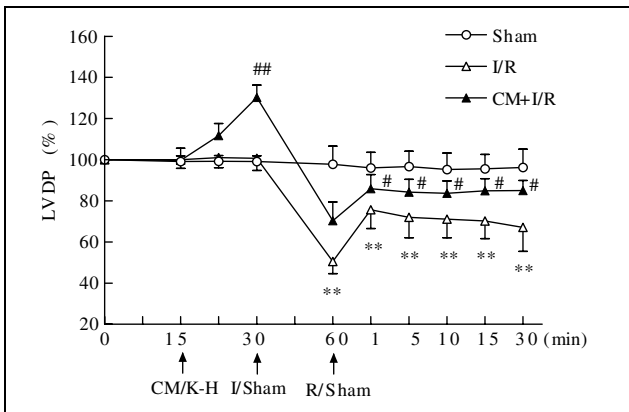


Fig. 2: Effect of CM (0.5 g/L) on the LVDP of isolated perfused rat hearts during ischemia and reperfusion (I/R). Values are expressed as $\bar{x} \pm s$, n = 12. * P < 0.05, **P < 0.01 compared with the control group (sham); # P < 0.05, ## P < 0.01 compared with the I/R group

parameters markedly (p < 0.05). In Fig. 2, the alteration of LVDP, the main parameter of contractility, was presented.

2.3. Effect of CM on contractility of isolated ventricular myocytes

Perfusion of the isolated ventricular myocytes with CM (0.25 g/L to 1.0 g/L) led to the increase in the peak velocity of cell shortening/relengthening ($\pm dL/dt_{max}$) and the amplitude of contraction (dL) in a dose-dependent manner. However, it did not alter the end-diastolic length (L0) of isolated myocytes (P > 0.05) (Fig. 3).

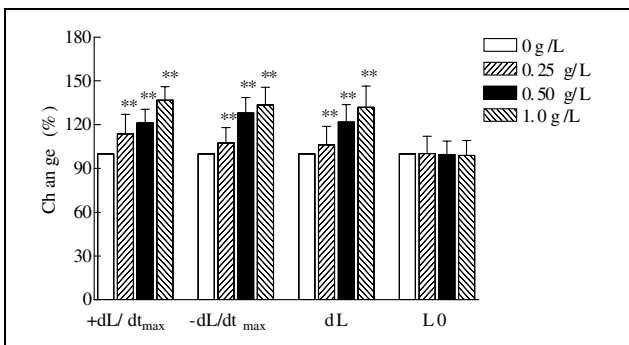


Fig. 3: Effect of CM on contractile parameters in isolated ventricular cardiomyocytes (% of control). Values are expressed as $\bar{x} \pm s$, n = 15. **P < 0.01 compared with control (0 g/L)

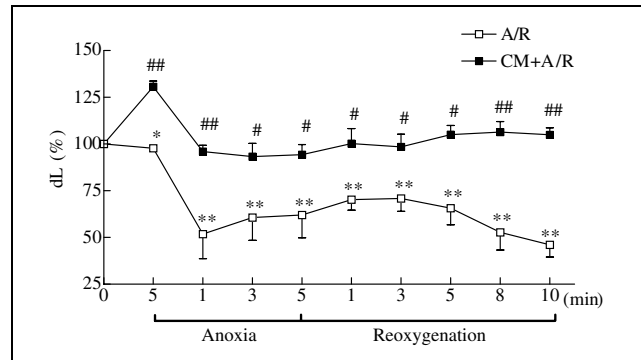


Fig. 4: Effect of CM (0.5 g/L) on dL during anoxia and reoxygenation in the isolated rat ventricular myocytes. Values are expressed as $\bar{x} \pm s$, n = 8. *P < 0.05, **P < 0.01 compared with normoxia; # P < 0.05, ## P < 0.01 compared with anoxia/reoxygenation (A/R) group

2.4. Effect of CM on contraction in isolated ventricular myocytes during anoxia and reoxygenation

Fig. 4 shows that the cell contraction amplitude (dL), the main parameter of contractility in single myocytes, decreased during anoxia, and then showed transient recovery followed by a progressive decrease during reoxygenation.

3. Discussion

In the present study, *Chrysanthemum morifolium* Ramat.(CM) increased LVDP and $\pm dp/dt_{max}$ in the isolated rat heart dose-dependently, and enhanced the inotropic status of cardiac myocytes. Furthermore, CM improved the decrease of LVDP, $\pm dp/dt_{max}$ and CF induced by ischemia/reperfusion, and attenuated the reduction of myocyte contraction induced by anoxia/reoxygenation. The results suggest that CM could protect the myocardium against the injury induced by ischemia/reperfusion or anoxia/reoxygenation.

Recently, the single adult isolated cardiac myocyte has become a popular experimental preparation, which has been used in numerous studies to define the mechanical, electrophysiological and biochemical properties of myocytes in the absence of diffusion-limiting extracellular spaces and endogenous myocardial neurohormones (Duthinh and Houser 1988). At the same time, a variety of methods for the measurement of cell motion have been developed, including laser diffraction techniques, photodiode arrays and video motion detectors. Edge-detection techniques are commonly used, but are limited due to their failure to automatically track a target myocyte that moves. Furthermore, the relatively high price of these devices limits access for scientists in developing countries. The system used in the present study is a computer-based video tracking system with powerful automatic tracking and recognition features, cheaper than those available commercially, and developed by our team. The contraction parameters of cardiac myocytes under normoxic conditions were comparable to those reported by other laboratories (Mohabir and Lee et al. 1991; Wang and Wang et al. 2000).

In the present study, CM not only increased the CF in normal isolate rat heart, but also attenuated the CF change induced by ischemia/reperfusion. In a recent study we found that CM caused endothelium-dependent relaxation in rat aortic rings and increased the activity of superoxide dismutase and decreased the content of malonic dialde-

hyde of the isolated rat heart suffered from ischemia/reperfusion. So the cardioprotection of CM may be mediated by its vasorelaxation and antioxidation. Further studies are needed to identify the underlying mechanisms.

4. Experimental

4.1. Material

The flower of *Chrysanthemum morifolium* Ramat. (CM) used in this research is called Hangbaiju. It was purchased from Tongxiang Cunfa Hangbaiju Product Company, Zhejiang Province, PR China and identified by associate professor Juanhua Xu (Institute of Chinese Traditional Medicine, Zhejiang University, PR China).

Male Sprague-Dawley rats (230–250 g) were obtained from the Experimental Animal Center of the Chinese Academy of Medical Sciences.

4.2. Preparation of extract

The dried and powdered flowers of CM were extracted twice with distilled water at 90 °C for 1.5 h, the combined filtrate was concentrated in vacuo below 55 °C on a rotary evaporator to 0.25 g/ml. The pH and relative density (compared with the same volume of water) of the concentrated extract was 5.36 and 1.04 respectively; the total content of flavonoids was 11.7 mg/ml.

4.3. Preparation and perfusion of isolated rat heart

A male Sprague-Dawley rat was executed. The heart was rapidly removed and placed in 4 °C modified Krebs-Henseleit solution (KH in mmol/L: 118.0 NaCl, 4.7 KCl, 1.2 K₂PO₄, 1.2 MgSO₄, 25.0 NaHCO₃, 1.25 CaCl₂, 10.0 glucose). The aorta was cannulated and the heart was perfused in Langendorff apparatus with KH solution at 37 °C, saturated with 95% O₂/5% CO₂ mixed gas. The perfusion pressure was maintained at 76 mmHg. A fluid-filled latex balloon was introduced through the mitral valve into the left ventricle. The balloon was connected via a short plastic tube to another pressure transducer and a computer for measurement of the left ventricular developed pressure (LVDP), heart rate (HR), and maximal rate of rise/fall of ventricular pressure (\pm dP/dt_{max}). Ischemia was achieved by the ligation of the left descending coronary artery for 30 min as the method of Shekher (Shekher and Singh 1997), and followed by 30 min reperfusion after release of the ligation.

4.4. Measurement of contractility in isolated ventricular myocytes

Isolated adult ventricular myocytes from a male SD rat were obtained by enzymatic dissociation (Farmer et al. 1983). After 1.5 h of stabilization, the isolated myocytes were moved to a 2.5 ml chamber perfused with modified KH solution with 1% BSA. A mixed gas phase of 95% O₂/5% CO₂ was maintained and KH solution was continuously delivered to the chamber by a constant flow apparatus (2 ml/min). The plexiglass chamber was equipped with a clear glass bottom and mounted on the stage of a microscope (IX80, Olympus). Attached to the microscope sideport was a charge-coupled device camera for video imaging of cells in the chamber. Myocyte contractions were elicited by field stimulation at a frequency of 0.5 Hz, and intensity twice the contraction threshold. A video-tracking system was used to measure the peak velocity of cell shortening ($+dL/dt_{max}$), the peak velocity of cell relengthening ($-dL/dt_{max}$), the amplitude of contraction (dL) and the end-diastolic length (L₀) of the isolated myocytes (Suzuki et al. 1998).

4.5. Experimental protocols

4.5.1 Effect of CM on cardiac function of the isolated rat heart

Isolated rat hearts were perfused with KH solution for 15 min until equilibrium. Hearts in the control group were perfused with KH solution for

30 min, while hearts in the CM group received perfusion with KH solution containing CM 0.25 to 1.0 g/L for 15 min followed by KH solution for 15 min.

4.5.2 Effect of CM on cardiac contractility of the isolated rat heart during ischemia/reperfusion

In the sham group, rat hearts were experienced sham operation and perfused for 75 min. Rat hearts in the ischemia/reperfusion (I/R) group were perfused for 15 min, then the left anterior descending coronary artery (LAD) was ligated for 30 min, followed by 30 min reperfusion. In the CM + I/R group, rat hearts were pretreated with CM at 0.5 g/L for 15 min before I/R.

4.5.3 Dose-related effect of CM on contraction of ventricular myocytes

After 5 min of stabilization, the ventricular myocytes were treated with CM accumulatively from 0.25 g/L to 1.0 g/L.

4.5.4 Effect of CM on contraction in isolated ventricular myocytes during anoxia and reoxygenation

Ventricular myocytes were subjected to 5 min of anoxia followed by 10 min of reoxygenation. In the DM group, myocytes were perfused with CM (0.5 g/L) for 5 min, then subjected to anoxia and reoxygenation in the presence of CM.

4.6. Data analysis and statistics

Data were expressed as means \pm se. Statistical significance were determined by the Student's t-test. Difference were considered significant when $p < 0.05$.

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