

## Protective effect of hyperin against myocardial ischemia and reperfusion injury<sup>1</sup>

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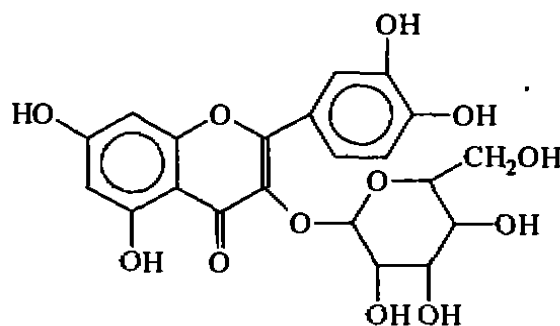
**KEY WORDS** quercetin; myocardial reperfusion injury; hemodynamics; creatine kinase; lactate dehydrogenase; cations; lipid peroxidation; malondialdehyde

**AIM:** To study the protective and antiperoxidative effects of hyperin (hyperoside; quercetin-3-O-galactoside; Hyp) on myocardial ischemia/reperfusion. **METHODS:** The rabbit anterior descending branch of left coronary artery was occluded for 60 min and then released to allow reperfusion for 20 min. Hemodynamics (LVP, LV  $\pm dp/dt$ ) and electrocardiogram (ECG, lead II) were monitored continuously with polygraph. After reperfusion, the blood sample and myocardium were taken to assay plasma creatine phosphokinase (CPK), lactate dehydrogenase (LDH), and cations in myocardium. Using a Langendorff system, the isolated heart of rat was initiated by ischemia for 40 min followed by 30 min of reperfusion. Malondialdehyde (MDA) contents of cardiac effluent and myocardium were measured with fluorescence spectrophotometer. **RESULTS:** Hyp 10 mg·kg<sup>-1</sup> iv depressed changes in LVP, LV  $\pm dp/dt_{max}$ , ECG, plasma CPK, LDH, and cations (Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>) in myocardium induced by ischemia/reperfusion in rabbits. Hyp 10 and 100  $\mu\text{mol}\cdot\text{L}^{-1}$  markedly reduced the increase in MDA production in isolated rat hearts after ischemia/reperfusion. **CONCLUSION:** Hyp possesses a protective effect against myocardial ischemia/reperfusion injury via attenuating lipid peroxidation.

The major hypotheses explaining the cellular events involved in myocardial post-ischemic reperfusion injury are calcium (Ca) overload and

free radical formation. Experimentally, various Ca channel blocking agents can enhance myocardial preservation<sup>(1,2)</sup>. But evidences that Ca antagonists lessen reperfusion-induced myocardial damage remain inconclusive<sup>(3)</sup>.

Hyperin (hyperoside; quercetin-3-O-galactoside; Hyp) was reported to block Ca influx in nervous and cardiovascular system<sup>(4,5)</sup>. The present study was to investigate the effects on cardiac dysfunction and peroxidation associated with ischemia/reperfusion *in vivo* and *in vitro*.



3, 3', 4', 5, 7-Pentahydroxyflavone-3-O-galactoside

### MATERIALS AND METHODS

**Materials** Hyp (Anhui Institute of Medical Science); thiobarbituric acid (TBA) (Shanghai Second Reagent Factory).

**Ischemia/reperfusion in rabbit hearts** Rabbits ( $n = 6$ ) of either sex weighing  $2.3 \pm 0.3$  kg were randomly assigned into 5 groups: 4 groups were pretreated with normal saline, Hyp 2.5, 5, and 10 mg·kg<sup>-1</sup>. A sham-operation group served as normal control.

A catheter was inserted into the left ventricle via carotid artery. LVP, LV  $\pm dp/dt$ , and lead II ECG were monitored continuously with 4-channel polygraph. The anterior descending branch of left coronary artery was occluded for 60 min followed by 20 min of reperfusion<sup>(6)</sup>. Hyp was injected (iv bolus) 5 min before ischemia and 60 min before reperfusion, respectively. At the end of reperfusion, the samples of blood and left ventricular myocardium were taken to assay plasma LDH, CPK, and contents of cations.

**LDH and CPK assay** Using Beckman LDH and Trace

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CK kits, plasma LDH and CPK were assayed with automatic biochemistry analyser (Beckman 700, USA)<sup>7,8</sup>.

**Cation assay** Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>-</sup>, and K<sup>+</sup> were measured with atomic absorption spectrophotometer (P-E 703, USA)<sup>(9)</sup>.

**Ischemia/reperfusion in isolated rat hearts** Wistar rats (*n* = 6) of either sex weighing 262 ± 34 g were randomly divided into 5 groups: normal control (full oxygen), ischemia/reperfusion, Hyp 1, 10, and 100 μmol·L<sup>-1</sup> groups.

The heart was perfused with O<sub>2</sub>-containing Ringer-Locke solution via aorta using a Langendorff system at 38 °C. Ischemia initiated by suspending perfusion for 40 min was followed by 30 min of reperfusion<sup>(11,6)</sup>. Hyp was added into the perfusion fluid 15 min before ischemia and throughout reperfusion. The cardiac effluent was collected during reperfusion and myocardium was homogenized.

**MDA assay** Using TBA method, MDA was measured with fluorescence spectrophotometer (Hitachi 650, Japan) at λ<sub>ex</sub> 515 nm, λ<sub>em</sub> 553 nm<sup>(10,11)</sup>.

**Statistics**<sup>(12)</sup> We used *t* test for measurement data and exact probability test for enumeration data.

**RESULTS**

**Hemodynamic changes induced by ischemia/reperfusion** LVSP, LV ± dp/dt<sub>max</sub> in control decreased during ischemia and further declined during reperfusion. Hyp prevented these changes (Fig 1).

**ECG changes induced by ischemia/reperfusion** There were no ECG changes in sham-operation group and the heart rate (HR) was 245 ± 29 bpm. Post-ischemic reperfusion caused the incidences of T-wave change, ST-segment changes, transmural Q-wave and arrhythmia to increase to 100 %, 100 %, 83.3 %, and 83.3 %, respectively (*vs* sham group, *P* < 0.05), and the HR to decrease to 205 ± 27 bpm (*vs* sham group, *P* < 0.05). Hyp 10 mg·kg<sup>-1</sup> iv reduced the incidences of ST-segment change, transmural Q-wave and arrhythmia to 16.7 %, 0 %, and 0 %, respectively (*vs* control, *P* < 0.05), and increased the HR to 240 ± 26 bpm (*vs* control, *P* < 0.05). The effect on the incidences of T-wave change (50 %) was insignificant (*vs* control, *P* > 0.05).

**LDH and CPK release after reperfusion** Post-ischemic reperfusion produced an increase in cellular enzyme release. The plasma LDH and CPK elevations were inhibited by Hyp treatment (Tab 1).

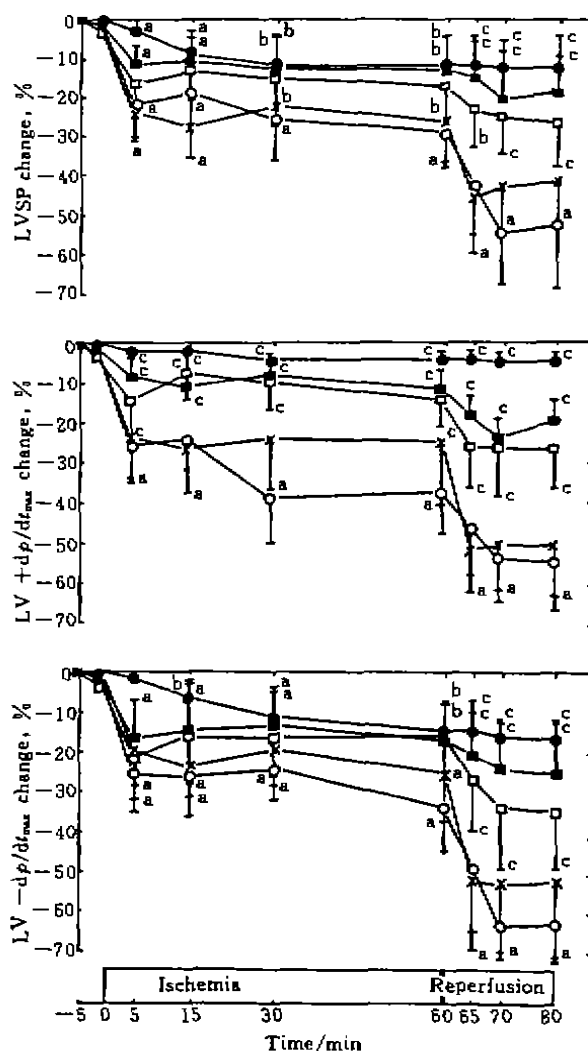


Fig 1. Effect of Hyp on hemodynamic changes during ischemia/reperfusion. Control (○), sham (●), Hyp 2.5 (×), 5 (□), 10 (■) mg·kg<sup>-1</sup>. *n* = 6 rabbits,  $\bar{x} \pm s$ . \**P* > 0.05, <sup>b</sup>*P* < 0.05, <sup>c</sup>*P* < 0.01 vs (○).

Tab 1. Effects of Hyp on plasma LDH and CPK in reperfusion injured rabbits. *n* = 6,  $\bar{x} \pm s$ . \**P* > 0.05, <sup>c</sup>*P* < 0.01 vs control.

Group	Dose/mg·kg <sup>-1</sup>	LDH/IU·L <sup>-1</sup>	CPK/IU·L <sup>-1</sup>
Control	-	611 ± 166	3 473 ± 285
Hyp	2.5	549 ± 55 <sup>a</sup>	3 185 ± 795 <sup>a</sup>
Hyp	5.0	332 ± 82 <sup>b</sup>	2 843 ± 808 <sup>a</sup>
Hyp	10.0	319 ± 115 <sup>c</sup>	1 715 ± 546 <sup>c</sup>
Sham	-	220 ± 93 <sup>c</sup>	1 670 ± 548 <sup>c</sup>

**Tab 2. Effect of Hyp on cation contents in rabbit myocardium injured by reperfusion.**  
*n* = 6,  $\bar{x} \pm s$ . <sup>a</sup>*P* > 0.05, <sup>b</sup>*P* < 0.05, <sup>c</sup>*P* < 0.01 vs control.

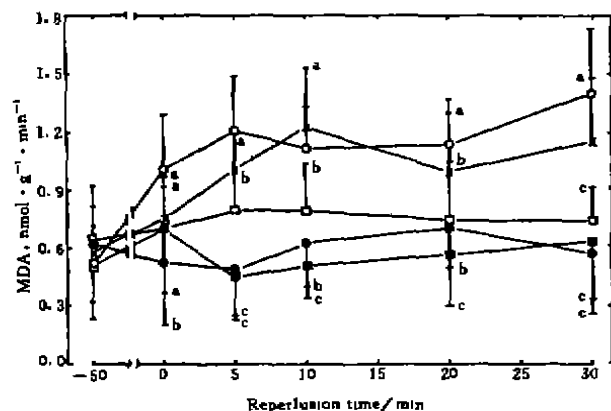
Group	Dose/mg·kg <sup>-1</sup>	Ca <sup>2+</sup> /ppm	Mg <sup>2+</sup> /ppm	Na <sup>+</sup> /ppm	K <sup>+</sup> /ppm
Control	-	161 ± 68	193 ± 35	1 363 ± 343	1 587 ± 600
Hyp	2.5	97 ± 59 <sup>a</sup>	138 ± 24 <sup>a</sup>	987 ± 316 <sup>a</sup>	1 463 ± 123 <sup>a</sup>
Hyp	5.0	73 ± 21 <sup>b</sup>	221 ± 44 <sup>a</sup>	918 ± 175 <sup>b</sup>	1 923 ± 569 <sup>a</sup>
Hyp	10.0	42 ± 10 <sup>c</sup>	263 ± 55 <sup>b</sup>	733 ± 137 <sup>a</sup>	1 895 ± 549 <sup>a</sup>
Sham	-	33 ± 19 <sup>c</sup>	262 ± 31 <sup>b</sup>	617 ± 61 <sup>a</sup>	2 128 ± 196 <sup>a</sup>

**Cation contents in myocardium after reperfusion** Myocardial Ca<sup>2+</sup> and Na<sup>+</sup> increased while Mg<sup>2+</sup> decreased upon reperfusion. These changes were attenuated by Hyp injection (Tab 2).

**MDA production in isolated rat hearts** Ischemia/reperfusion resulted in MDA increase in cardiac effluent and in myocardium. The increase in MDA production was markedly attenuated by Hyp infused (Tab 3, Fig 2).

**Tab 3. Effects of Hyp on MDA contents in rat myocardium injured by ischemia/reperfusion.** *n* = 8,  $\bar{x} \pm s$ .  
<sup>a</sup>*P* > 0.05, <sup>b</sup>*P* < 0.05, <sup>c</sup>*P* < 0.01 vs ischemia/reperfusion.

Group	$\mu\text{mol} \cdot \text{L}^{-1}$	MDA, nmol/g wet wt
Ischemia/reperfusion	-	189 ± 48
Hyperin	1	140 ± 23 <sup>a</sup>
Hyperin	10	120 ± 35 <sup>b</sup>
Hyperin	100	117 ± 15 <sup>c</sup>
Normal control	-	114 ± 8 <sup>c</sup>



**Fig 2. Effect of Hyp on MDA contents in cardiac effluent of reperfusion injured rat hearts.** Ischemia/reperfusion (○), normal control (●), Hyp 1 (×), 10 (□), 100 (■)  $\mu\text{mol} \cdot \text{L}^{-1}$ .  $\bar{x} \pm s$ . <sup>a</sup>*P* > 0.05, <sup>b</sup>*P* < 0.05, <sup>c</sup>*P* < 0.01 vs (○).

## DISCUSSION

Although the nature of myocardial post-ischemic reperfusion injury remains controversial with many hypotheses. In animal models there are 2 main mechanisms: Ca<sup>2+</sup> overload and free radical formation<sup>[3]</sup>. It seemed that there was some relationship between these 2 phenomena<sup>[13,14]</sup>. Also, the protective and antiperoxidative action of Ca blockers on reperfusion injury have been extensively reported<sup>[1,2]</sup>. Our results demonstrated that Hyp, a Ca<sup>2+</sup> channel blocking agent, produced an effect on reperfusion in facilitating the recovery of the mechanical and electrical functions of the heart. The protective effect on membrane damage was also evident from the reduction in reperfusion-induced cellular enzyme release.

In order to further elucidate the mechanism of the effect, an indirect index of oxygen free radical formation and lipid peroxidation signified by MDA was investigated. The results supported the hypothesis that protective effect of Ca channel blocker against reperfusion damage may be mediated by the inhibition of free radical and hence of lipid peroxidation in myocardium<sup>[14]</sup>. More substantial and direct proofs are required to clarify the mechanism.

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341-344

金丝桃苷对心肌缺血与再灌注损伤的拮抗作用<sup>1</sup>

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关键词 槲皮素; 心肌再灌注损伤; 血液动力学; 肌酸激酶类; 乳酸脱氢酶; 阳离子; 脂质过氧化; 丙二醛 金丝桃苷

A 目的: 观察金丝桃苷(Hyp)对缺血与再灌注心肌的保护作用及抗氧化作用。方法: 结扎家兔冠脉左降支 60 min 后松开结扎 20 min, 以多道生理记录仪持续记录左室内压(LVP)、左室内压变化速率(LV±dp/dt)、心电图(ECG)变化。再灌注后, 取血和左心室肌测血浆肌酸磷酸激酶(CPK), 乳酸脱氢酶(LDH)及心肌阳离子含量。采用Langendorff系统, 离体大鼠心脏缺血 40 min 后再灌注 30 min。以荧光分光光度计测定冠脉流出液和心肌组织丙二醛(MDA)的含量。结果: Hyp 10 mg·kg<sup>-1</sup> iv 可抑制缺血与再灌注所致的家兔 LVP、LV±dp/dt<sub>max</sub>、ECG、血浆 CPK、LDH 和心肌 Ca<sup>2+</sup>、Mg<sup>2+</sup>、Na<sup>+</sup> 含量的变化。Hyp 10 和 100 μmol·L<sup>-1</sup> 可降低缺血与再灌注所致离体大鼠心肌 MDA 含量的增高。结论: 金丝桃苷对心肌缺血与再灌注具有保护作用, 此作用可能与其抗脂质过氧化有关

R285.5 R965

全国第 6 届心血管药理学术会议将于明年召开

全国第 6 届心血管药理学术会议将于 1997 年 8 月下旬在北京召开。会议将邀请心血管研究领域著名专家作有关学术前沿的专题报告, 组织几个大家普遍关心问题的圆桌研讨会, 并同时举办几个实验技术讲习班。会议还将举行换届选举。欢迎全国从事心血管药理、生理、生化的研究工作者以及心血管临床医师参加。有意参加者请与北京医科大学第三医院血管医学研究所(邮编 100083)韩启德联系, 到时将寄给第二轮通知。

(中国药理学会心血管药理专业委员会)