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Prevention of global myocardial reperfusion injury on isolated rabbit hearts with furyl–dihydropyridines I

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ABSTRACT In the isolated rabbit heart of recirculating nonpulsatile perfusion circuit, furyl–dihydropyridines I 20 μmol · L^{-1} greatly reduced the leakage of myocardial enzymes and the concentration of malondialdehyde (MDA) in plasma, decreased the myocardial calcium and sodium contents, maintained normal coronary vascular resistance and prevented reperfusion arrhythmias of global postischemic reperfusion hearts. Its mechanism of protecting the ischemic–reperfused myocardium might be associated with the diminution of calcium influx of myocardial cells and cellular lipid peroxidation induced by oxygen free radicals.

KEY WORDS calcium channel blockers; dihydropyridines; myocardial reperfusion injury; lipid peroxides; calcium; sodium; potassium

Oxygen–free radicals are believed to participate in myocardial ischemic and reperfusion injury[1,2]. They occur as a consequence of the retroduction of molecular oxygen into the previously ischemic tissue and accumulation of intracellular calcium overload which promotes conversion of xanthine dehydrogenase to xanthine oxidase during reperfusion. These substances may cause membrane damage leading to local electrophysiological derangements that trigger serious ventricular arrhythmia[3,4]. The furyl–dihydropyridines I (2,6–dimethyl–4–furyl–1,4–dihydropyridine–3,5–dicarboxylate) was proved in our previous study[5] to have an antiarrhythmic effect. The aim of our present study is to investigate whether this drug can protect against lipid peroxidation and prevent myocardial postischemic reperfusion damages in isolated rabbit hearts.

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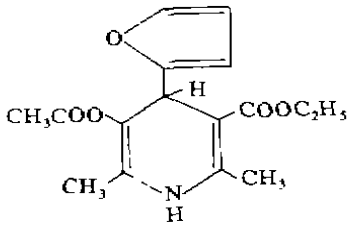

Furyl–dihydropyridines I

MATERIALS AND METHODS

Isolated heart model and perfusion A recirculating nonpulsatile perfusion circuit was used[6]: The heart–lung machine was composed of an LDB–H electropump (Ding Shan Instrument Factory, Zhejiang) and a bubble oxygenator which incorporates the functions of oxygenation, defoaming, heat exchange, blood storage and filtration. The supplementary apparatus included glass cover of constant temperature bath and an air flow–meter (Suzhou Chemical Instrument Factory, Jiangsu).

Healthy adult white rabbits (♂, weighing 2.1 ± SD 0.4 kg) were anesthetized with sodium pentobarbital 20 mg · kg^{-1} iv and given heparin 5 mg · kg^{-1} iv. After exposure by median sternotomy, the heart was rapidly excised and placed in Ringer lactate solution at 4℃ (CH_3COONa 74.6, NaCl 100, KCl 4.0, $CaCl_2$ 3.0 mmol · L^{-1}). Retrograde aortic perfusion with 37℃ oxygenated, diluted autogenous blood (30 ml of Ringer lactate solution, 3 ml of 4% $NaHCO_3$ and 50 ml of autogenous blood) was started immediately after cannulation of the assenting aorta. The mean perfusion pressure was maintained at

8.0 / 5.3 kPa (60 / 40 mm Hg). pO_2 ((49.2 ± 17.5 kPa), pCO_2 (5.3 ± 1.2 kPa), and pH (7.4 ± 0.1) of the reperfusates was kept within the physiological range; the mean corpuscular volume being more than 20%.

This experiment was divided into 2 groups: furyl-dihydropyridines I 20 μmol · L^{-1} or 0.2% Tween 80 (solvent control group) of equal volume was injected into the perfusion circuit 3 min prior to global ischemia (decreased perfusion pressure to 1 / 6 of initiation). Thirty minutes later, reperfusion was initiated by restoring blood flow to normal for 60 min. Two tiny electrodes were placed on the apex of the heart and the root of the aorta, and an epicardiogram was made by means of a multipurpose polygraph (Nihon kohden, Japan).

Analysis of effluent enzymes and mearurement of malondialdehyde (MDA) Blood samples taken from the heart-lung machine before ischemia served as a normal value before ischemia; and those samples collected from the coronary venous sinus at 5, 15, 30, and 60 min of reperfusion were analyzed for creatine phosphokinase (CPK) and α- hydroxybutyrate dehydrogenase (HBD) activities by the Multistat-III Analyzer (Beckman company, USA). The concentration of MDA was measured according to the colorimetric method of thiobarbituric acid.

Measurements of calcium, sodium, potassium of myocardium At the end of the experiment, about 1 g of myocardial tissue isolated from the left ventricle (ischemic tissue) was dried at 100-105℃ for 5 h, and then was dissolved in 5 ml of HNO_3-$HClO_4$ of 4 : 1; calcium, sodium, and potassium contents were determined by an atomic absorption spectrophotometer (Hitachi 180-80, Japan); sodium was measured at wavelength of 589.6 nm; potassium at 766.9 nm; and calcium at 422.7 nm.

Coronary flow (CF) and coronary resistance (CR) Under the constant pressure perfusion, the CF and the CR were calculated according to the Poiseuille's law[6].

Drug Furyl-dihydropyridines (I), obtained from instructor ZHANG Yan-Hong and Professor WU Qi, Department of Chemistry, Shanxi Teachers University, and was dissolved in Tween 80 and double distilled water[5].

Statistics All the measurements were expressed as $\bar{x} \pm SD$ and were statistically evaluated by t test, and coefficient of correlation was also evaluated.

RESULTS

Enzymes released from the myocardium during reperfusion The values of CPK, HBD were not significantly different between the solvent control group and the drug (furyl-dihydropyridines I 20 μmol · L^{-1}) group ($P > 0.05$, $n = 6$). As reperfusion continued, the amount of CPK release at 5, 15, 30, 60 min and HBD release at 15, 30, 60 min increased in control group ($P < 0.01$ *vs* preischemia respectively, Fig 1).

At 5 and 15 min of reperfusion, the CPK level in the drug group was significantly less than that in the solvent control group ($P < 0.01$, Fig 1) at 30, 60 min of reperfusion, CPK and HBD in the drug group were also significantly less than those in the solvent control group ($P < 0.05$ or 0.01 respectively, Fig 1).

CF and CR Under the constant pressure perfusion, CF in the solvent control group gradually decreased, hence CR increased ($P < 0.01$, $n = 6$, Fig 1). However, CF and CR value were unchanged ($P < 0.05$ *vs* preischemia, $n = 6$, Fig 1) in the drug group. Compared with the control group, CF in the drug group was relatively increased and therefore, CR was reduced significantly when measured at 30 and 60 min of reperfusion ($P < 0.05$ or 0.01, Fig 1).

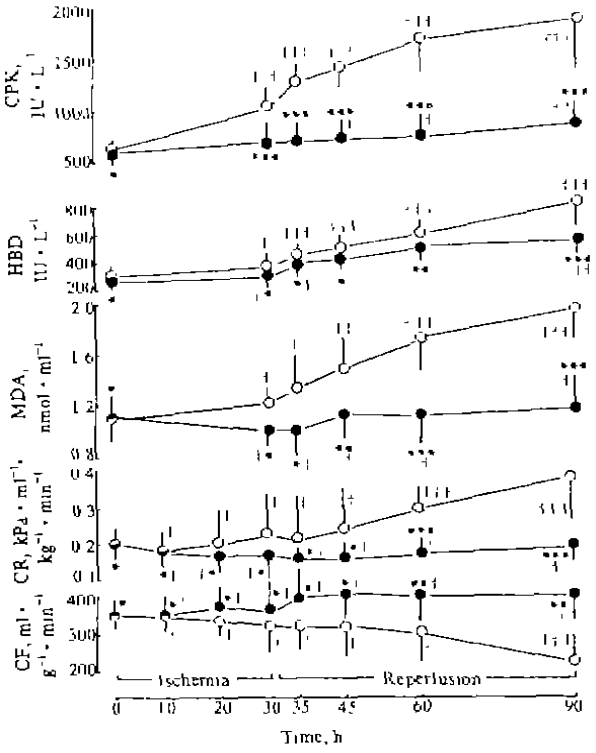

Fig 1. Effects of furyl-dihydropyridines I on activities of creatine phosphokinase (CPK), α-hydroxybutyrate dehydrogenase (HBD), coronary resistance (CR), coronary flow (CF), and malondialdehyde (MDA) content in isolated rabbit hearts during global ischemia and reperfusion. (○) solvent control (0.2% Tween 80), (●) furyl-dihydropyridines I 20 μmol · L⁻¹. $n=6$, $\bar{x}\pm SD$. $^{+}P>0.05$, $^{++}P<0.05$, $^{+++}P<0.01$ *vs* preischemia; $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* solvent control.

Effect on concentration of MDA In the solvent control group, the concentration of MDA in blood samples gradually increased at 15, 30, and 60 min of reperfusion ($P<0.05$ or 0.01 *vs* preischemia, $n=6$ respectively, Fig 1). The concentration of MDA in the drug group was significantly less than that in the control group at 15, 30, and 60 min of reperfusion ($P<0.05$ or 0.01, Fig 1). Furyl-dihydropyridines I 20 μmol · L^{-1} decreased the concentration of MDA in isolated global postischemic reperfusion rabbit hearts.

Correlation of MDA content and CPK release The content of MDA and CPK was remarkedly increased during reperfusion the MDA content correlated with CPK release ($r=0.964$, $P<0.01$). Furyl-dihydropyridines I 20 μmol · L^{-1} inhibited the content of both. This change of both groups was correlative ($r=0.917$, $P<0.01$). It shows that the amount of CPK release was related to the degree of lipid peroxidation.

Effects on myocardial Na, K, Ca content Furyl-dihydropyridines I 20 μmol · L^{-1} decreased myocardial Na, Ca content ($P<0.01$ *vs* the solvent control group, Tab 1) and could not effect on myocardial K content ($P>0.05$ *vs* the solvent control group, Tab 1).

Tab 1. Effects of furyl-dihydropyridines I (FDP I) on calcium content (μmol · g^{-1} dry wt), sodium and potassium contents (mmol · g^{-1} dry wt) of postischemic reperfusion myocardium in isolated rabbit hearts. $n=6$, $\bar{x}\pm SD$. $^{**}P<0.05$, $^{***}P<0.01$ *vs* solvent (0.2% Tween 80).

	Solvent	FDP I
Calcium	10.72 ± 1.50	6.23 ± 1.75**
Sodium	1.49 ± 0.17	1.16 ± 0.17**
Potassium	0.21 ± 0.04	0.28 ± 0.05*

Effects on reperfusion arrhythmias In the solvent control group, ventricular premature beats (VPB) were 5/7 during coronary artery ligation; VPB, ventricular tachycardia (VT), ventricular fibrillation (VF) and cardiac arrest (CA) of reperfusion were 7/7, 6/7, 2/7, and 2/7 respectively. Injection of furyl-dihydropyridines I 20 μmol · L^{-1} reduced the incidence of ischemia and reperfusion arrhythmia, the incidence of both was 1/7 ($P<0.01$ of VPB, $P<0.05$ of VT *vs* the solvent control group).

DISCUSSION

The changes of the content of MDA reflect indirectly the amount of free radical formation[4]. Oxygen radicals can react with membrane lipids to form unstable lipid peroxides, which can lead to membrane injury and resultant cell death[7]. Myocardial cell enzyme release is a marker of myocardial reperfusion injury[8]. Reperfusion or reoxygenation of the myocardium is associated with an increased calcium influx[9], and the removal of calcium from the prefusate before a period of ischemia or hypoxia[11] delays the development of reperfusion–induced arrhythmia and improves recorvery[11]. Our results show that furyl–dihydropyridines I may decrease myocardial calcium and sodium accumulation and increase myocardial potassium content.

Pridjian[12] suggested that ischemia and reperfusion injury lead to stepwise increase in myocardial sodium, calcium content. It is possible that injury induced by ischemia and reperfusion opens the calcium slow channels to sodium[13], allowing inward sodium flux. When myocardial intracellular potassium decreased by 50%, Na–Ca exchange reduced 68%, the correlation between K / Na value and intracellular Ca content was negative in ischemic myocardium[14]. The salutary effect of slow channel blocking agents in the postischemic heart may thus involve prevention of entry of both sodium and calcium[12].

In conclusion, furyl–dihydropyridines I used before ischemia protects the myocardium against some of the mechanical and metabolic damage caused by postischemic reperfusion. Its mechanism of protecting the ischemic–reperfused heart might involve diminution of the cellular lipid peroxidation induced by oxygen free radicals, decrease of myocardial calcium accumulation and maintenance of normal coronary vascular resistance and thereby limitation of myocardial injury during global ischemia and reperfusion in isolated rabbit hearts.

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呋喃二氢吡啶 I 对离体兔心全心缺血再灌注损伤的保护作用

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提要 离体兔心全心缺血 30 min，再灌注 1 h，使心肌损伤逐渐加重。呋喃二氢吡啶 I 20 μmol · L^{-1} 能明显抑制缺血心肌肌酸磷酸激酶、α-羟丁酸脱氢酶、丙二醛的释放量；减少缺血复灌心肌钙、钠含量；降低缺血复灌心肌冠脉阻力的增加，预防再灌注心律失常的发生。提示该药保护心肌与减少缺血心肌钙含量及脂质过氧化程度有关。

关键词 钙通道阻滞剂；二氢吡啶类；心肌再灌注损伤；脂质过氧化；钙；钠；钾

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Effects of isocorydine on action potentials in isolated canie Purkinje fibers and ventricular muscles[1]

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ABSTRACT Standard microelectrode techniques were used to study the effects of isocorydine (Isoc) on potential characteristics of canine cardiac Purkinje fibers (PF) and ventricular myocardium (VM) *in vitro*. In PF, the action potential durations (APD), APD_{50}, and APD_{90}, were prolonged at 3 μmol · L^{-1} but shortened at 30 μmol · L^{-1} by Isoc. The action potential amplitude (APA) and the maximal upstroke velocity (V_{max}) were decreased at 100 μmol · L^{-1}. In VM, the action potential characteristics were changed by Isoc at above 30 μmol · L^{-1}. APD_{50} was shortened but APD_{90} was prolonged. V_{max} were decreased at 30 μmol · L^{-1}. The effective refractory period (ERP) was prolonged by Isoc in PF and VM. The results suggest that Isoc may interfere with K^+, Na^+, and Ca^{2+} currents in myocardiac cell membrane at different concentrations.

KEY WORDS isocorydine; Purkinje fibers; myocardium; action potentials

Isocorydine (Isoc), an alkaloid contained in many plants including *Dactylicapnos scandens* (Hutch) and *Dicranostigma leptopodum* (Maxim) Fedde, possesses a potent anti-arrhythmic effect[1]. Electrophysiological study has shown that Isoc 30 μmol · L^{-1} reduced the APA of rabbit sinoatrial node and the spontaneous electical activity induced by Ba^{2+} ion in guinea pig VM[2]. It was considered that Isoc may inhibit the slow calcium inward current. The purpose of this study was to elucidate the effects of Isoc on action potentials of canine PF and VM to reveal the basic mechanism of its anti-arrhythmic effect.

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