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Effects of tetrahydroberberine on ischemic and reperfused myocardium in rats

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ABSTRACT The effects of tetrahydroberberine (THB) on ischemic and reperfused myocardium were studied in comparison with verapamil (Ver). In anesthetized rats, THB and its analogues, *l*-THP and *l*-SPD, reduced the infarct size after 4 h of left anterior descending coronary artery (LAD) ligation. In Langendorff hearts, in common with Ver, THB 1 and 10 μmol·L^{-1} markedly decreased the incidences of ventricular tachycardia (VT) and ventricular fibrillation (VF) in the reperfusion period. The malondialdehyde content and xanthine oxidase activity were also decreased in global ischemic-reperfused hearts pretreated with THB ($P<0.01$, or $P<0.05$). It suggested that THB could protect the myocardium from ischemic and reperfusion injury.

KEY WORDS berberine; verapamil; myocardial infarction; myocardial reperfusion injury; free radicals

Tetrahydroberberine (THB), *l*-tetrahydropalmatine (*l*-THP), and *l*-stepholidine (*l*-SPD) are analogues of tetrahydroprotoberberines, which could be used clinically as analgesics. THB, *l*-THP, and *l*-SPD have similar effects on the cardiovascular system, such as anti-arrhythmia[1-3], hypotension[4], and myocardial infarct reduction[5]. In this paper, compared with verapamil, THB was investigated in ischemia / reperfusion injury with coronary artery ligation and with the Langendorff heart.

MATERIALS AND METHODS

THB, *l*-THP, and *l*-SPD, supplied by the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, were dissolved in a slightly acidic solution. Rats were bred from the Laboratory Animal Center of Jiangsu Province.

Myocardial infarct size (MIS) Sprague-Dawley rats, weighing 235± *s* 42 g, were anesthetized by ether inhalation. The heart was exposed and the LAD immediately ligated[6]. Each drug solution (1.5 mmol·L^{-1}) was injected 30 min before ischemia into the sublingual vein at a dosage of 10 ml·kg^{-1} (equivalent to 5.1, 5.3, and 4.8 mg·kg^{-1} of THB, *l*-THP, and *l*-SPD, respectively), and Ver 1.7 mg·kg^{-1}. The heart was removed 4 h after LAD occlusion. The ventricles were cut horizontally into 3-4 sections and immersed in nitro blue tetrazolium solution (pH 7.4, 37℃) for 10 min. The infarct myocardium was dissected carefully from the normal tissue, blotted, and weighed. The % of infarct myocardium in the left ventricle was calculated[7].

Reperfusion arrythmias in Langendorff heart Rats, ♂, weighing 267± *s* 14 g, were anesthetized with ip sodium pentobarbital 35 mg·kg^{-1}. The heart was excised, flushed with heparinized saline (20 IU·ml^{-1}), and perfused by the Langendorff method with modified Krebs-Henseleit solution (containing Na_2EDTA 0.5 mmol·L^{-1}) aerated with 95% O_2 + 5% CO_2 at 37℃. The coronary perfusion flow was regulated to 6-8 ml·min^{-1}. After 15-20 min equilibration, the LAD was ligated. After 15 min, the knot was cut to allow reperfusion. During ischemic period and the first 3 min of reperfusion, an epicardial electrogram was recorded by inserting a stainless steel wire into the myocardium at the apex as one electrode and attaching the another to the root of aorta. Drugs or solvents were added into the K-H solution 10 min before ischemia until the end of the experiment.

Malondialdehyde (MDA) production and xanthine oxidase (XOD) activity in myocardium On Langendorff hearts, the global ischemia and

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reperfusion model was formed as follows. 1) Equilibration for 20 min: the heart was paced at 4 Hz (10 ms pulse-width, twice threshold voltage) until the end of experiment with a stimulator and a bipolar steel electrode in contact with the right ventricle. 2) "Ischemic" perfusion for 40 min: coronary flow was reduced to 0.3–0.4 ml · min^{-1}. The heart was mounted horizontally in a closed heat-preserved trough maintaining the temperature at 37℃. 3) "Reperfusion" for 15 min: the coronary flow was reinstated to 6–8 ml · min^{-1}. Hearts in the normal perfusion group were perfused for 75–80 min while the coronary flow was maintained at 6–8 ml · min^{-1}.

At the end of the experiment, the ventricles were promptly excised from the heart, weighed, and chilled in ice-cold saline. Tissue homogenates were prepared in a ratio of 1 g wet tissue to 10 ml of saline by using a homogenizer XHF-1 for 60 s, and then centrifuged at 1000× g for 10 min. The supernatant was used for assays. Colorimetry methods were used to determine protein[8], MDA[9], and XOD[10].

RESULTS

Effects of THB, *l*-THP, and *l*-SPD on MIS THB, *l*-THP, and *l*-SPD reduced the MIS after LAD ligation for 4 h in rats ($P<0.01$ *vs* solvent). No significant differences were found between the drugs ($P>0.05$, Tab 1).

Tab 1. Effects of terahydroberberine (THB) and its analogues, *l*-tetrahydropalmatine (*l*-THP) and *l*-stepholidine (*l*-SPD), on myocardial infarct size (MIS) after 4 h of coronary artery ligation in anesthetized rats. $\bar{x}\pm s$. $^{*}P>0.05$, $^{***}P<0.01$ *vs* **solvent. LV: left ventricle. Ver= verapamil.**

Drug	*n*	LV wt / mg	Infarct wt / mg	MIS / %
saline	16	606± 54	226± 32	37± 3*
solvent	10	585± 61	204± 42	35± 4
THB	10	611± 55	167± 37	27± 4***
l-THP	14	603± 70	156± 44	26± 6***
l-SPD	9	593± 67	162± 57	27± 4***
Ver	10	622± 64	157± 35	25± 4***

Effect of THB on reperfusion arrythmia inLangendorff rat hearts During 15 min of LAD ligation, 7 perfused hearts showed a slight decrease and 8 an increase in heart rate ($P>0.05$); 6 of them had sporadic ventricular premature contractions. After reperfusion, 8 of the 9 solvent-perfused hearts developed typical VF immediately after cutting the ligation-knot and lasted >3 min in 5 cases. Both THB 10 μmol · L^{-1} and Ver 0.1 μmol · L^{-1} pretreated hearts were capable of abolishing the reperfusion-induced VT and VF perfectly ($P<0.01$, Tab 1). THB 1 μmol · L^{-1} reduced the incidence of VT and VF ($P<0.01$), and prolonged their latency ($P<0.05$, Tab 2).

Tab 2. Effects of THB and verapamil (Ver) on arrythmias induced by coronary artery ligation for 15 min followed by reperfusion for 5 min in Langendorff hearts of rats. $\bar{x}\pm s$. $^{**}P<0.05$, $^{***}P<0.01$ *vs* **control.**

Drug / μmol · L^{-1}	*n*	Ventricular Incidence / %	Tachycardia Latency / s	Fibrillation Latency / s
Control	9	88.9	6± 5	6± 5
THB 1.0	6	33.3***	23± 23**	28± 29**
THB 10.0	6	0***	–	–
Ver 0.1	6	0***	–	–

Effects of THB on production of free radicals in ischemic and reperfused rat hearts Tab 3 demonstrated that in the solvent group with global ischemia

Tab 3. Effects of THB and Ver on myocardial malondialdehyde (MDA) production and xanthine oxidase (XOD) activity in the global ischemic and reperfused rat hearts. $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **solvent.**

Drug, μmol · L^{-1}	*n*	MDA, μmol / g protein	XOD, IU / g protein
Normal	7	6± 3***	38± 26***
Solvent	10	23± 11	101± 38
THB 20.0	9	9± 4***	46± 16***
THB 10.0	8	18± 12*	70± 19**
Ver 0.1	7	4.8± 2.5***	63± 22**

(40 min) and reperfusion (15 min), the MDA content and XOD activity were 2.7 and 1.7 times higher, respectively, than those in normal perfused hearts ($P<0.01$). Ver 0.1 $\mu mol \cdot L^{-1}$ and THB 20 $\mu mol \cdot L^{-1}$ reduced MDA production and inhibited XOD activity in comparison with the solvent group ($P<0.01$, or $P<0.05$). Pretreatment with THB 10 $\mu mol \cdot L^{-1}$ suppressed the XOD activity ($P<0.05$), but did not diminish the MDA to any significent extent.

DISCUSSION

The extent of myocardial cell necrosis and ventricular arrhythmia during acute myocardial infarction (AMI) are important determinants of mortality as well as subsequent congestive heart failure. Therefore, some of the efforts to reduce the mortality after AMI have been directed at decreasing the infarct size and combating the arrhythmia. In anesthetized dogs, THB and its anologues (*l*-THP, *l*-SPD) could increase myocardial blood flow and lessen coronary resistance and cardiac oxygen consumption[4]. These effects were beneficial to myocardium. Meanwhile, it was shown in this study that THB could protect the myocardium against reperfusion-induced damage in the isolated rat heart, providing further evidence that THB could be a useful agent in AMI treatment concomitant with thrombolytic therapy.

Impaired energy metabolism, oxygen free radical generation, and calcium overloading have been postulated to be the probable factors in the mechanism of reperfusion injury. XOD was considered the major cause of free radical damage in myocardial ischemia and reperfusion[11]. Some proteinases which catalyze xanthine dehydrogenase to form XOD were activated by excessive Ca^{2+} in reperfused myocardium. Thereby, the XOD activity and the content of MDA fomation (an end product of lipid peroxidation) increased significantly. Through blocking of the Ca^{2+} influx across the sarcolemma, Ver could prevent calcium overloading, and inhibit the transformation of XOD from xanthine dehydrogenase.

THB was reported to inhibit 80 $mmol \cdot L^{-1}$ KCl-stimulated ^{45}Ca influx markedly in smooth muscles of guinea pig *taenia coli*[12]. But whether THB, like Ver, had a role against ischemia and reperfusion as a calcium antagonist remains to be further studied.

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四氢小檗碱对大鼠缺血及再灌注心肌的作用

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摘要 四氢小檗碱 (THB) 及其同类物左旋四氢巴马汀、左旋千金藤立定能明显缩小麻醉大鼠心脏冠脉结扎 4 h 后的梗塞范围. 在 Langendorff 心脏, THB 与维拉帕米能显著降低再灌注心律失常的发生率, 延长其潜伏期, 并降低再灌注心肌中丙二醛含量及黄嘌呤氧化酶活力. 提示 THB 具有保护缺血及再灌注心肌作用.

关键词 小檗碱; 维拉帕米; 心肌梗死; 心肌再灌注损伤; 自由基

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Methylflavonolamine protects aorta from atherosclerosis in cholesterol-fed rabbits

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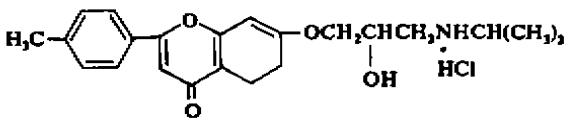
ABSTRACT The effect of methylflavonolamine [4′-methyl-7-(2-hydroxy-3-isopropylamino-propoxy)-flavone hydrochoride, MFA], synthesized recently by the Shanghai Institute of Pharmaceutical Industry, on the development of atherosclerosis was studied in male New Zealand white rabbits fed cholesterol for 10 wk. MFA, 7 mg·kg^{-1} daily ip, did not significantly alter the serum total cholesterol, HDL, and triglyceride levels, but significantly lowered the aortic cholesterol and calcium contents. Atheromatous lesions covered 53.3± 11.8% of the intimal surface of the aorta in the saline group and 11.3± 2.3% in the MFA group ($P<0.01$). We conclude that MFA suppresses cholesterol-induced atherosclerosis.

KEY WORDS flavonolamine; atherosclerosis; aorta

Methylflavonolamine, [4′-methyl-7-(2-hydroxy-3-isopropylamino-propoxy)-flavone hydrochoride, MFA], a compound recently synthesized by the Shanghai Institute of Pharmaceutical Industry, has anti-arrhythmic effects[1,2], increases coronary blood flow, and prevents experimental infarction[3]. MFA inhibits the contraction induced by extracellular calcium in rabbit isolated aortic strip[4]. It is postulated that MFA might interfere with calcium availability. Since compounds which interfere calcium availability inhibit experimental atherosclerosis at large dosage[5-8], MFA might aalso interfere with the development of atherosclerosis. The present experiment was designed to study the effects of MFA on serum total cholesterol, aortic cholesterol and calcium deposition, and plaque formation in cholesterol-fed rabbits.



4′-methyl-7-(2-hydroxy-3-isopropyl-amino-propoxy)-flavone hydrochloride (methylflavonolamine, MFA)

MATERIALS AND METHODS

New Zealand ♂ white rabbits ($n=28$) weighing 1.98± s 0.09 kg were housed individually and randomly assigned to 3 groups: 1) standard pellets and ip saline (standard group, $n=8$), 2) cholesterol

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