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.

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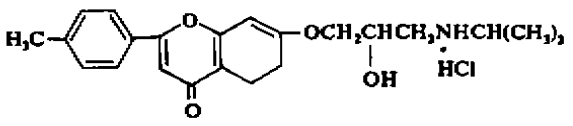

4′-methyl-7-(2-hydroxy-3-isopropropyl-amino-propoxy)-flavone hydrochloride (methylflavonolamine, MFA)

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

pellets providing cholesterol 1 g daily per rabbit and ip saline (saline group, $n=10$), 3) cholesterol pellets providing cholesterol 1 g daily per rabbit and ip MFA 7 mg· kg^{-1} daily (MFA group, $n=10$). The rabbit in the cholesterol-fed groups was first fed with 50 g 2% cholesterol pellets and then another 50 g standard pellets to make sure that the rabbit consumed 1 g cholesterol daily. Water was provided ad lib. MFA powder, supplied by the Shanghai Institute of Pharmaceutical Industry, was dissolved in distilled water before use.

At 0, 5, and 10 wk, blood was collected from the central ear artery. The serum was analyzed for total cholesterol, HDL, triglyceride, and total protein with automatic analyser (Gilford, USA). Serum Na, Ca, K, and Mg were assayed by atomic absorption spectrophotometer (Shimadzu, Japan).

At the end of 10 wk feed period, the rabbits were exsanguinated under pentobarbital anesthesia. The whole aorta, trimmed off the connective tissue, was opened longitudinally. The intima areas covered by atheromatous plaques were measured without staining by planimetry from an enlarged (× 2) color photograph. The aorta was weighed and cut into small pieces. The minced aorta was delipidated with 3 changes of 10 ml chloroform-methanol 2 : 1 (vol : vol), 60 min each, followed by 2 changes each of acetone and ether[9]. The supernatant fluids from each extraction were pooled and evaporated. The residue was redissolved in glacial acetic acid to determine cholesterol colorimetrically with phthalaldehyde[10]. The delipidated aorta was stored in a dessicator at room temperature until the weight no longer reduced. The dried, defatted aortic tissue was dissolved in 2 ml 56.5% HNO_3, boiled, and then diluted to 10 ml with distilled water for the determination of calcium. Differences between the groups were evaluated by t test.

RESULTS

Effects on body weight and serum constituents One rabbit in the standard group, 1 in the saline group and 2 in MFA group died of unknown cause during the feed period. Two rabbits in the saline group became paralysed in their hind legs in the 6th wk and died in the 8th and 10th wk, respectively. Values for body weight and serum biochemical analyses at the end of 10 wk were summarized in Tab 1. The body weight of the MFA group was lower than those of the standard and the saline groups. The serum total protein level of the saline group was higher than those of the standard and the MFA groups.

Effects on serum lipid levels Serum total cholesterol levels tended to be lower in the MFA group as compared with the saline group ($P>0.05$). Serum triglyceride in the saline group, but not in the MFA group, was higher than that in the standard

Tab 1. Body weight and plasma constituents. $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$, *vs* **standard group.** $^{+}P>0.05$, $^{++}P<0.05$ *vs* **Saline group.**

	Standard group	Saline group	MFA group
Rabbits	7	7	8
Body weight / kg	2.95± 0.16	2.82± 0.15*	2.55± 0.09^{**++}
Calcium / mmol· L^{-1}	3.01± 0.08	2.97± 0.19*	2.91± 0.11^{*+}
Sodium / mmol· L^{-1}	131.3± 2.6	125.5± 2.9*	128.3± 0.8^{*+}
Postassium / mmol· L^{-1}	4.76± 0.22	5.80± 0.51*	5.02± 0.25^{*+}
Magnesium / mmol· L^{-1}	1.65± 0.15	1.64± 0.29*	1.54± 0.18^{*+}
Total protein / g· L^{-1}	82.4± 2.4	93.2± 2.1**	84.5± 1.8^{++}

group. HDL in MFA group, not in the saline group, was higher than that in the standard group (Tab 2).

Effects on aorta Gross and microscopical examinations revealed that fibrous-fatty plaques on the aortic intima were severer in the saline group than in the MFA group. Fibrous fatty plaques were thicker around the openings of artery branches in both groups. Extensive fibrous fatty plaques on the intima of the arch and compactly scattered plaques in the thoracic segment were found in the saline group. Scattered fibrous fatty plaques in the arch, and rarely scattered plaques in the thoracic portion were detected in the MFA group. The lesions on the intima in the MFA group was significantly lower than that in the saline group (Tab 3). The extent of aortic atheromatous lesions was nil in the standard group.

The total cholesterol and calcium contents of the aorta in the MFA group were significantly lower than those in the saline group, but higher than those in the standard group (Tab 3).

DISCUSSION

The present study showed that MFA markedly reduced cholesterol-induced lesion in the aorta, while the serum cholesterol level was not significantly lowered. These results indicate that MFA protect aorta from atherosclerosis induced by elevated serum cholesterol. Calcium deposition in the aorta is considered one of important determinants in the progression of atherosclerosis[5]. MFA considerably suppressed the excessive accumulation of calcium and deposition of cholesterol in the aorta, suggesting that

Tab 2. Serum levels of lipids ($g \cdot L^{-1}$). $n = 7$, $\bar{x} \pm s$. $^{*}P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* **standard group.** $^{+}P > 0.05$ *vs* **Saline group.**

	0 wk	5 wk	10 wk
Total cholesterol			
Standard group	0.39± 0.05	0.43± 0.13	0.38± 0.09
Saline group	0.36± 0.04*	16.8± 1.4***	17.7± 1.9***
MFA group	0.38± 0.08^{*+}	14.2± 1.0^{***+}	14.5± 1.2^{***+}
HDL cholesterol			
Standard group	0.21± 0.03	0.21± 0.06	0.22± 0.02
Saline group	0.24± 0.04*	0.28± 0.05*	0.36± 0.06*
MFA group	0.18± 0.02^{*+}	0.37± 0.03^{**+}	0.44± 0.06^{**+}
Triglyceride			
Standard group	1.57± 0.24	1.05± 0.42	0.93± 0.22
Saline group	1.68± 0.24*	1.85± 0.24*	2.25± 0.45**
MFA group	1.88± 0.34^{*+}	2.07± 0.25^{*+}	1.35± 0.18^{*+}

Tab 3. Aortic total cholesterol, calcium, and intimal lesion ratio. $\bar{x} \pm s$. $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* **standard group.** $^{++}P > 0.05$, $^{+++}P < 0.01$ *vs* **Saline group.**

	Standard group	Saline group	MFA group
Rabbits	7	7	8
Cholesterol, mg / g dry defatted wt	20± 4	138± 28***	63± 7^{***++}
Calcium, μg / g dry defatted wt	444± 26	884± 86***	560± 40^{**++}
Plaque covered area, %	0	53.3± 11.8***	11.3± 2.3^{***+++}

MFA delayed the progression of atherosclerosis. However, it is uncertain whether the reduction in aortic calcium is the cause or result of the anti-atherosclerotic effect of MFA.

MFA is comparably potent in inhibiting the smooth muscle contraction induced by physiological agonists, but is rather weak in inhibiting the contraction induced by the Ca influx through the potential dependent Ca channels as compared with the well known Ca channel blockers[4,11,12]. The present result showed that MFA effectively suppressed the atherogenesis at same range of dosage as Ca channel blockers used for the same purpose. Other actions, besides the inhibition of Ca influx, may be also involved in the anti-atherogenesis of MFA. Antiplatelet aggregation[13] and inhibition of gastroenteric function[11] may also contribute to the anti-atherogenic effect of MFA.

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甲基黄酮醇胺抑制喂胆固醇所致的兔动脉粥样硬化

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摘要 本文观察了甲基黄酮醇胺(MFA)对喂胆固醇所致兔动脉粥样硬化的影响. 实验时间为 10 wk. 结果为: ip MFA 7 mg· kg^{-1}· d^{-1} 对血浆总胆固醇，HDL 和甘油三脂无明显影响，但使主动脉胆固醇和钙含量显著降低. MFA 组的主动脉内膜粥样斑覆盖率为 11.3%，低于生理盐水组 53.3% ($P<0.01$). 本研究证明 MFA 抑制喂胆固醇所致的兔动脉粥样硬化.

关键词 黄酮醇胺; 动脉粥样硬化; 主动脉