相对的稳定, 使得细胞成为一种 "永生化 "状态, 因此, 端粒酶活性的增加与肿瘤的发生、发展密切相关; 临床资料表明造血系统的恶性肿瘤普遍表现出端粒酶活性增强[8], 其中以急性白血病端粒酶活性最强, 在白血病的不同阶段其端粒酶水平也不完全一样, 端粒酶活性的高低与白血病的进展程度相一致, 复发及难治性急性白血病的端粒酶水平明显高于缓解及恶性程度较低的急性白血病, 端粒酶活性越高的白血病患者其化疗效果及预后越差, 存活率也越低。因此, 端粒酶活性的高低对于判断白血病的疗效、恶性程度、预后及复发等均具有重要的临床意义。

端粒酶活性的变化与细胞凋亡的关系目前尚未完全阐明。近期研究资料显示, 在细胞凋亡过程中, 端粒酶可能起重要的调节机制[9,10], 端粒酶活性下降可以促进细胞的程序性死亡过程, 端粒酶活性抑制剂可以增加急性白血病细胞的凋亡率, 而且可以增加其他化疗药物的临床疗效, "抗端粒酶疗法 "有可能成为一种广泛的白血病治疗方法, 而且也是筛选新型抗肿瘤药物的重要指标之一。本实验结果显示, 冬凌草甲素可显著降低 Raji 细胞的端粒酶活性, 抑制细胞的生长, 诱导细胞发生凋亡, 这可能是冬凌草甲素体外抗白血病作用的重要机制之一。

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Effects of crocetin on experimental atherosclerosis in rats

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Abstract: **Object** To study the effect of crocetin (CCT) on experimental atherosclerotic formation in rats. **Methods** Atherosclerotic rat models were replicated by administration of excessive vitamin D_2 (VD_2) followed by feeding a high-cholesterol diet. The blood samples were assayed for the content of TC, TG, LDL-C, HDL-C, MDA level, and the activity of SOD in serum. The sections of aortia and liver were examined. **Results** High- and low-dosages of CCT were found significantly to reduce serum TC, LDL-C, and MDA level; and elevate serum HDL-C level, SOD activity, and antiatherosclerotic index (AAI) in atherosclerotic rats. The histopathological observation of aortic arch showed the alleviation of atherosclerotic damage by CCT. **Conclusion** CCT has obvious antiatherosclerotic effect in rats.

Key words: crocetin; antiatherosclerosis; blood lipid; vitamin D_2 (VD_2)

西红花酸对大鼠实验性动脉粥样硬化的影响

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摘 要: 目的 研究西红花酸对大鼠实验性动脉粥样硬化的影响。方法 采用给予大剂量维生素 D_2 (VD_2) 后饲喂

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高胆固醇饲料诱发大鼠实验性动脉粥样硬化模型,测定大鼠血清总胆固醇 (TC)、甘油三酯 (TG)、低密度脂蛋白 (LDL-C)、高密度脂蛋白 (HDL-C)、丙二醛 (MDA) 含量和超氧化物歧化酶 (SOD) 活性。取主动脉和肝脏做病理切片检查。结果 高、低剂量的西红花酸能显著降低动脉粥样硬化大鼠血清 TC、LDL-C 和 MDA 含量; 显著升高血清 HDL-C 含量、SOD 活性和抗动脉粥样硬化指数 (AAI)。病理切片结果表明, 西红花酸能明显减轻模型大鼠动脉粥样硬化性损伤。结论 西红花酸具有显著的抗大鼠实验性动脉粥样硬化作用。

关键词: 西红花酸; 抗动脉粥样硬化; 血脂; 维生素 D_2
中图分类号: R286.2 文献标识码: A 文章编号: 0253-2670(2004)07-0777-05

Introduction

Atherosclerosis, the principal cause of heart attack, stroke, and gangrene of the extremities, is responsible for 50% of all mortality of them in the USA, Europe, and Japan[1]. It is a disease of the arterial injury characterized by the accumulation of lipids, proliferation of smooth muscle cells, and calcification[2]. The animals such as rabbit, chicken, monkey, and pig can not be widely used as the atherosclerotic model because of various reasons. It is well known that the rat is not susceptible to atherosclerosis induced only by administration of high cholesterol diet (HCD)[1,3], but administration of excessive vitamin D_2(VD_2) and an antithyroid agent to rat in addition to high cholesterol diet results in formation of atheromatous lesions[4]. Whether crocetin (CCT), one of naturally occurring components in *Gardenia jasminoides* Ellis[5], which had hypolipidemic effect[6] and antioxidant properties[7-10], has antiatherosclerotic effect on this model has not been reported. Therefore the effect of CCT on atherosclerosis using this model was studied.

1 Materials and methods

1.1 Animals and reagents

Sprague-Dawley rats (weighing 200—250 g, purchased from Animal Experiment Center of China Pharmaceutical University) were housed in a room [(23 ± 1) ℃ and (60 ± 10) % humidity] under an artificial 12-hour light/dark cycle (7:00 a.m.—7:00 p.m.).

The composition (%) of HCD: standard chow (92.6), cholesterol (2.0), lard (5.0), cholic acid (0.2), propylthiouracil (0.2).

CCT, with purity of over 80% (HPLC), was extracted from *G. jasminoides* and dissolved in sodium carboxylmethylcellulose (CMC-Na); VD_2 was dissolved in the peanut oil; Simvastatin (Sim), product of Hangzhou Merk Co., Ltd., was formulated as water suspension.

1.2 Experimental protocol

Experimental atherosclerotic rat model was established according to the method of Morisaki[4] with some modification. After one week acclimatization, the rats were randomized into five groups of 10 animals each: normal group; model group (VD_2+ HCD); CCT (H) group (VD_2+ HCD+ 50 mg/kg CCT); CCT (L) group (VD_2+ HCD+ 25 mg/kg CCT); Sim group (VD_2+ HCD+ 4 mg/kg Sim). All rats except for normal group were treated by oral administration of 300 000 IU/(kg · d) of VD_2 for three consecutive days during which standard chow was given. Meanwhile CCT or Sim was orally given to the corresponding rats. From the fourth day, VD_2-treated rats consumed HCD for 6-week period in which CCT or Sim was continuously administrated. The rats were weighed every two days in order to adjust the dosage of CCT or Sim.

1.3 Biochemical parameters

Blood samples were collected from 12h-fasting animals by puncture in the retro-orbital sinus at the end of the experiment. Serum TC, LDL-C, HDL-C, triglyceride (TG) were analyzed enzymatically using Kit from Beijing Zhongsheng Bioengineering Company. Anti-atherosclerotic index (AAI) was calculated by the formula: AAI = HDL-C/(TC − HDL-C). Serum SOD and MDA were measured using Kit from Nanjing Jiancheng Bioengineering Company. Liver was weighed and liver index (LI) was defined as liver weight (g) per 100 g body weight.

1.4 Morphological parameter

Animals were killed at the end of the experiment, aortic arch and liver were fixed in Bouin's

solution, dehydrated, embedded in paraffin and cut at 5 mm. The sections were stained with hemalun-eosin (HE).

In aorta, atherosclerosis was scored as reported[11]. Focus was given to the main characteristics, such as accumulation of foam cells in interlamellar spaces, rectitude and fragmentation of medial elastic lamellae, abundance of collagen fibers, proliferated and disoriented smooth muscle cells surrounding a lipid-calcic core.

In liver, the severity of damage was defined as three degrees of heavy, moderate, and mild according to lipid droplets and apoptotic changes.

1.5 Statistical analysis

Data were expressed as $\bar{x} \pm s$ and compared with analysis of t-test.

2 Results

2.1 Blood lipids and lipoproteins

As shown in Table 1, the serum TC level in model group was about seven times that in normal group, of which LDL-C was 22-fold increased and HDL-C decreased by 40%. AAI was surprisingly lower and TG showed no significant change indicating that atherosclerosis model was established successfully. Compared with those in model group, TC and LDL-C in CCT (H) group were decreased by 40% and 55%; LDL-C and AAI increased by about 40% and 200%, respectively; TG remained no significant change. There was no difference between CCT (H) and CCT (L) groups. In this experiment, parameters in Sim group were similar with those in CCT groups.

Table 1 Effect of CCT on blood lipids and lipoproteins in experimentally atherosclerotic rats ($x \pm s$, n= 10)

Groups	Dosage/(mg · kg^{-1})	TC/(mmol · L^{-1})	LDL-C/(mmol · L^{-1})	HDL-C/(mmol · L^{-1})	TG/(mmol · L^{-1})	AAI
Normal	–	1.77±0.18	0.43±0.07	1.01±0.15	1.72±0.25	1.38±0.38
Model	–	12.32±3.16$^{\#\#}$	9.69±3.85$^{\#\#}$	0.66±0.21$^{\#\#}$	1.41±0.43	0.06±0.03$^{\#\#}$
CCT (H)	50	7.68±1.89**	4.14±1.90**	1.60±0.23**	1.74±0.68	0.18±0.07**
CCT (L)	25	7.08±2.59**	4.03±1.73**	1.04±0.27**	1.35±0.81	0.19±0.07**
Sim	4	8.18±2.14**	4.69±1.77**	0.95±0.15**	1.41±0.86	0.15±0.06**

$^{\#\#}P < 0.01$ *vs* normal group; $^{**}P < 0.01$ *vs* model group

2.2 Serum MDA and SOD

Rats in model group exhibited a higher content of MDA and a lower activity of SOD in serum than those in normal group. In CCT group and Sim group, the MDA and SOD were improved, even returned back to the normal level for CCT (H) group and Sim group (Table 2).

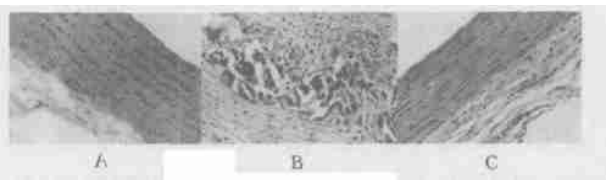
Table 2 Effect of CCT on serum MDA and SOD in experimentally atherosclerotic rats ($x \pm s$, n= 10)

Groups	Dosage/(mg · kg^{-1})	SOD/(U · mL^{-1})	MDA/(mmol · L^{-1})
Normal	–	167.49± 8.16	5.10±1.33
Model	–	158.85±16.22$^{\#}$	6.83±1.37$^{\#}$
CCT (H)	50	169.74±13.09*	5.15±1.51*
CCT (L)	25	166.68±10.60*	5.37±1.21*
Sim	4	168.51±20.00*	5.35±1.00*

$^{\#}P < 0.05$ *vs* normal group; $^{*}P < 0.05$ *vs* model group

2.3 Morphological alterations in atherosclerotic rats

2.3.1 Aortic lesions: Light microscopy showed no evident aortic lesions found in normal group (Fig. 1-A). In CCT group, none of the obvious aortic lesions could be seen as compared with the model group, featuring more accumulation of foam cells in interlamellar spaces, rectitude and fragmentation of medial elastic lamellae, abundance of collagen fibers, proliferated and disoriented smooth muscle cells surrounding a lipid-calcic core (Fig. 1-B). The atherosclerosis was score of 12.0±2.1 (Table 3), for model group, and 4.0±0.9 for CCT (H) group (Table 3). Fig. 1-C showed that no lipid deposition, foam cells, widening in intima and no proliferation of smooth muscle cell, no increased extracellular matrix and calcification in media. As were also in the Sim group.



A-normal group B-model group C-CCT group

Fig. 1 Morphological structure of aorta in atherosclerotic rats (×200)

2.3.2 Liver lesions and liver index: No abnormalities were observed in the liver of normal

group. However, rat in model group demonstrated the severe lipid droplets and apoptotic changes which were slightly alleviated in the CCT and Sim group.

Table 3 Effect of CCT on pathological score of aorta and liver index of experimentally atherosclerotic rats ($x \pm s$, n= 10)

Groups	Dosage /(mg · kg^{-1})	Liver index /(g · 100 g^{-1})	Pathological score of aorta
Normal	–	2.7±1.3	0
Model	–	4.7±0.3##	12.0±2.1##
CCT (H)	50	4.4±0.4*	4.0±0.9**
CCT (L)	25	4.4±0.5*	3.8±0.8**
Sim	4	4.6±0.4	3.3±0.5**

$P < 0.01$ *vs* normal group; * $P < 0.05$ ** $P < 0.01$ *vs* model group

As shown in Table 3, the raised liver index was elevated markedly in model group in comparison with normal group. However, this parameter was significantly reduced in CCT and Sim groups.

3 Discussion

Administration of excessive VD_2 followed by feeding HCD containing the antithyroid agent propylthiouracil caused severe hypercholesterolemia and atherosclerotic lesions in rat[4,11], of which propylthiouracil was recognized to produce a mild hypothyroidism in order to provide a more favorable environment for atherosclerisis development in rats. The role VD_2 played seemed to be mediated by the arterial calcification process in which myocytes serve as a local factor. It is induced by the increase of conjunctive constituents by calcification, production of calcifiable substances (glycoproteins and glucoaminoglycans), and modification in their own environmental physico-chemical conditions favourable to calcium precipitation[12].

In the present study, the morphological observation to aortic arch by quantifying the histopathologial change completely confirmed the successful establishment of the atherosclerotic model in rat. On this model, the antiathero-sclerotic effect of CCT was observed. Main changes representing the typical atherosclerosis, such as widening, foam cells formation, lipid deposition in intima and calcification, lipid deposition, smooth muscle cell proliferation in media were rarely found in CCT treated rat aorta compared with that in model group. Also on this model, the damage of liver in CCT groups was also less serious than that of model group in which severe lipid droplets and apoptotic changes were characterized. The smaller liver index in CCT group could be also explained by the fact that less lipid deposited in the liver.

Biochemically, this combined treatment increased the serum TC level, of which most were LDL-C with as much as 25 or 7 times that in the control level, meanwhile, decreased the HDL-C level significantly. However, serum TG remained unchanged. This was compatible to the report[3,4]. The above findings indicated that the formation of atherosclerotic lesions in rats, was mainly attributable to the high level of TC instead of TG. Both high LDL-C and low HDL-C level favored the atherosclerosis because of the poor AAI. Such disorders were implied in the atherogenesis[1] by accumulation of macrophages loaded with lipid (foam cell) derived from monocytes. CCT at both high and low doses, enjoyed its potentially antiatherosclerotic effect partly by significantly lowering serum TC and LDL-C, especially by elevating HDL-C which protects against the progression of atherosclerosis not only by transporting the cholesterol from foam cells in the arterial wall to the liver, but also by protecting the endothelium from injury[13] resulting in prevention of leukocyte and platelet adhesion and in maintenance of the anticoagulant properties of endothelium, and by inhibition of smooth muscle cell proliferation. These effects of CCT were similar to that of Sim, a HMG-CoA inhibitor.

Oxidative stress, specifically the oxidation of LDL, has long been suspected of having a critical role in the development of atherosclerosis, and antioxidants have been expected to have potential as antiatherogenic agents. As an antioxidant, CCT´s mechanism of antioxidation could partly explain its antiatherosclerotic effect by raising serum SOD and lowering serum MDA. However, the other mechanism of CCT´s effect on antiatherosclerosis is being further studied in our laboratory.

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麻黄汤及单味麻黄中麻黄碱与伪麻黄碱在小鼠组织中的药动学研究

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摘 要: 目的 分析麻黄汤 (HED) 和单味麻黄 (HE) 中麻黄碱 (ephedrine, E)、伪麻黄碱 (pseudoephedrine, PE) 在小鼠脑、肺、心、肝、肾的动态变化, 探讨 HED 配伍的意义。方法 用 GC-MS/SIM 法, HP-5 弹性石英毛细管 (25 m×0.2 mm), 载气 He, 无分流进样, 柱初温 120 ℃, 1 min 后以 10 ℃/min 升至 185 ℃, 再以 30 ℃/min 升至 245 ℃, 保持 5 min, 选择离子 (SIM, m/z = 154, 265), 进样量 1 μL。结果 建立了组织中 E、PE 的测定方法。HED 及 HE 中 E、PE 在各组织中的动力学变化不一致, 且 HED、HE 中 E 或 PE 在同一组织中的动力学参数亦不同。其中 E 在脑、肺、肾的分布量是 HE> HED。结论 本法稳定、可靠, 适于 E、PE 在组织中的含量测定。HED 中桂枝、杏仁、甘草影响了 E、PE 在小鼠组织的动力学过程。

关键词: 麻黄碱; 伪麻黄碱; 配伍; GC-MS/SIM; 药动学
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Pharmacokinetics of ephedrine and pseudoephedrine from *Herba Ephedrae* Decoction and *Herba Ephedrae* in mice tissues

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Abstract: **Object** To analyze the pharmacokinetics change of ephedrine (E) and pseudoephedrine (PE) in *Herba Ephedrae* Decoction (HED) and *Herba Ephedrae* (HE) in the brain, lung, heart, liver, and kidney of mice to discuss the meaning of compatibility of the prescription. **Methods** Gas chromatography-mass spectrometry with selected ion monitoring (GC-MS/SIM) was used. GC-MS conditions were: capillary column HP-5 (25 m×0.2 mm), Helium as carrier gas, non-split injection, column temperature 120 ℃ (1 min) $\xrightarrow{10\ ℃/min}$ 185 ℃ $\xrightarrow{30\ ℃/min}$ 245 ℃ (5 min), with 1.0 mL/min runoff, SIM (m/z = 154,

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