·药理实验与临床观察·

Vasodilatory effect of water decoction of dried flower of *Carthamus tinctorius* on rabbit aorta *in vitro*

LI Hong-fang[1], WANG Long-de[2], PANG Jin-jiang[1]*

(1. Department of Physiology, Lanzhou Medical College, Lanzhou 730000, China;
2. Affiliated Hospital, Gansu College of TCM, Lanzhou 730020, China)

Abstract: Object To observe the vasodilatory effect of a water decoction of the dried flower of *Carthamus tinctorius* L. (DFCT) on rabbit thoracic aorta and its mechanism. **Methods** Strips of rabbit aortic smooth muscle were suspended in organ baths containing Kreb's solution, and then isometric tension was measured. **Results** DFCT did not change the resting tension of rabbit aortic strips. But similar to Ach (10^{-5} mol/L), DFCT (20 mg/mL) could cause an obvious relaxation in 10^{-6} mol/L NA-precontracted arterial strips. The relaxant effect of DFCT was significantly reduced by removal of endothelium and 10^{-4} mol/L *L*-NNA and 10^{-5} mol/L methylene blue but not by prostaglandin synthase inhibitor and blockage of adrenergic β receptor. In addition, DFCT (40 mg/mL) inhibited NA and KCl cumulative concentration-response curves of aortic strips without endothelium, and changed the PD_2 values for NA from (6.06±0.09) in control group to (5.07±0.08) and for KCl from (1.71±0.33) in control group to (1.35±0.20), respectively. **Conclusion** These results suggest that the vasodilatory effect of DFCT in rabbit thoracic aorta may be related to the nitric oxide release from endothelium, and also probably due to inhibition of Ca^{2+} influx through receptor-operated and voltage-dependent calcium channels.

Key words: the dried flower of *Carthamus tinctorius* L.; isolated aortic strip; endothelium cells; calcium influx

红花水煎剂对家兔离体主动脉血管的舒张作用

李红芳[1], 汪龙德[2], 庞锦江[1]

(1. 兰州医学院 生理教研室, 甘肃 兰州 730000; 2. 甘肃中医学院附属医院, 甘肃 兰州 730020)

摘 要: 目的 观察红花 *Carthamus tinctorius* (DFCT) 水煎剂对血管肌条的舒张作用及机制。方法 将家兔离体主动脉肌条放置于灌流肌槽中, 记录其等长收缩。结果 DFCT 对血管肌条静息张力无明显影响, 但 20 mg/mL DFCT 水煎剂与 10^{-5} mol/L 乙酰胆碱相似, 可使 10^{-6} mol/L 去甲肾上腺素预收缩血管肌条产生明显的舒张作用。去除内皮细胞、10^{-4} mol/L *L*-NNA 或 10^{-5} mol/L 甲烯蓝可减弱 DFCT 的舒张血管作用, 但前列腺素合成抑制剂和β 肾上腺素能受体阻断无明显影响。另外, 40 mg/mL DFCT 水煎剂可明显抑制去内皮血管肌条去甲肾上腺和 KCl 的量效收缩反应, 使其 PD_2值分别由对照组 6.06±0.09 和 1.71±0.33 变为 5.07±0.08 和 1.35±0.20。结论DFCT 水煎剂可通过受体操纵 Ca^{2+} 通道和电压依赖性 Ca^{2+} 通道抑制外 Ca^{2+} 内流, 使血管肌条舒张, 其作用与内皮释放的 NO 有关。

关键词: 红花; 离体血管肌条; 内皮细胞; 钙内流
中图分类号: R286.59 文献标识码: A 文章编号: 0253-2670(2003)05-0430-04

The dried flower of *Carthamus tinctorius* L. (DFCT) is a traditional Chinese medicine. Its different preparations exhibit a wide diversity of biologic activities including anti-coagulation[1], anti-inflammation[2], antitumor activities[3]. Their anti-ischemia[4], antioxidative and fatigue-resisting proper-

* 收稿日期: 2002-09-08
基金项目: 甘肃省科委中青年科技基金资助项目(YW991-A23-011)
作者简介: 李红芳(1966—), 女, 甘肃省镇原县人, 1992 年毕业于兰州医学院生理学专业, 获医学硕士学位, 2002 年毕业于兰州大学生命科学学院生物学专业, 获理学博士学位。现为兰州医学院生理教研室副教授, 主要从事平滑肌生理与药理研究。

ties[5], their enzymatic inhibitory[6] and uterus excitory[7] effects have also been reported. Decoctions of DFCT have been studied in the cardiovascular system. Here a weak inhibitory effect on the heart of anesthetized animals and a decrease in blood pressure[8], an increase in coronary blood flow and the vasodilatory effect on the precontracted aorta[9] have been reported, but the vasoconstriction effect on peripheral blood vessels in the ear has also been found[10]. Therefore, the effect of water decoctions of DFCT on vascular smooth muscle and its mechanism are not completely clear. In this study, we have examined the mechanism of action involved in the vascular smooth muscle effect of DFCT on rabbit thoracic aorta.

1 Materials and methods

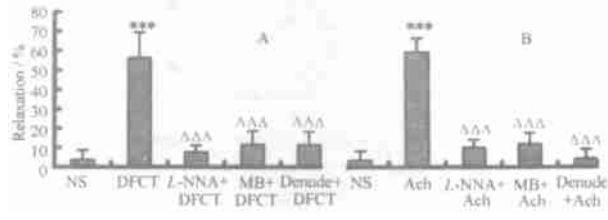
1.1 Drugs. DFCT (extracted and identified by Medicine Inspection Institute of Gansu Province, China) was smashed into pieces, decocted in water and made it into 100% concentration of decoction (g/mL); N-*L*-nitro-arginine (*L*-NNA, Sigma), indomethacin (Indo) (Taicang, Jiangsu Province, China) was dissolved in Na_2CO_3 solution and the pH was adjusted to 7.4; propranolol (Prop) (The Second Pharmaceutical Factory of Beijing, China); noradrenaline (NA, Datong Huida, China); methylene blue (MB, Merck); acetycholine (Ach, Sigma).

1.2 Arterial tension studies. Rabbits and arterial strips were prepared according to our previous study[11]. Isometric tension was recorded with BL-410 Experimental System of Biological Function (TME, China) through an IBM computer. After 90 minutes of equilibration, different experiments were carrid out: (1) in some experiments, DFCT was added in progressively increasing cumulative concentrations (10, 30 and 70 mg/mL) in order to observe its effect on the resting tension of aortic strips. (2) To analyze if DFCT could relax an existing contraction, arteriae with or without endothelium were contracted by NA (10^{-6} mol/L). When the contractile response had reached a stable plateau (approximately 5—10 minutes) DFCT (20 mg/mL) or Ach (10^{-5} mol/L) was added respectively. (3) To evaluate the possible mechanism of relaxation induced by DFCT in NA-contracted aortic strips, the strips with endothelium were contracted by NA and the response to DFCT or Ach was determined repeatedly after preincubation 15 minutes with one of the following substances: 10^{-4} mol/L *L*-NNA, 10^{-5} mol/L MB, 10^{-5} mol/L Indo and 10^{-5} mol/L Prop. (4) In additional experiments, after strips were stabilized under 2 g resting tension for 90 minutes in Krebs' solution, the concentration-response curve to NA ($10^{-7.5}$—10^{-5} mol/L) or KCl (10—100 mmol/L) was observed in the absence and presence of DFCT (40 mg/mL) in rabbit aortic strips without endothelium.

1.3 Data analysis. All the results were expressed as $\bar{x} \pm s$. Relaxation was expressed as percentage relaxation of contraction induced by NA (10^{-6} mol/L). In experiments of the concentration-response curves, the results were expressed as percentage of control contractile response induced by 10^{-5} mol/L NA and 100 mmol/L KCl respectively. Statistical analysis was performed in the paired and unpaired student's *t*-test. Comparison among multiple groups was made by analysis of variance (ANOVA).

2 Results

2.1 Effects of DFCT on resting tension and NA-precontraction in rabbit aortic strips. DFCT (10, 30 and 70 mg/mL) administrated in progressively increasing cumulative concentrations did not affect arterial resting tension. However, DFCT (20 mg/mL) (Fig. 1 A) and Ach (10^{-5} mol/L) (Fig. 1 B) caused (56.15 ± 3.85)% and (62.70 ± 2.27)% relaxation of NA-precontracted strips ($P < 0.001$, $n = 12$) respectively.



*** $P < 0.001$ *vs* NS; △△△ $P < 0.001$ *vs* DFCT

Fig. 1 Effects of *L*-NNA, MB, and Denude on relaxation induced by DFCT (20 mg/mL) (A) and Ach (10^{-5} mol/L) (B) in NA (10^{-6} mol/L) precontracted isolated rabbit aortic strips

2.2 Effects of *L*-NNA, MB, denuded endothelium (Denude), Indo and Prop on responses to DFCT or

Ach in NA-precontracted strips. Incubation with *L*-NNA (10^{-4} mol/L) or MB (10^{-5} mol/L) significantly reduced relaxation induced by DFCT or Ach in rabbit aortic strips ($P < 0.001$, $n = 10$). Endothelium removal also markedly decreased the relaxation ($P < 0.01$, $n = 10$, Fig. 1 A for DFCT and Fig. 1 B for Ach). However, incubation with Indo (10^{-5} mol/L) or Prop (10^{-5} mol/L) did not affect relaxation induced by the two preparations ($P > 0.05$, $n = 10$, Fig. 2).

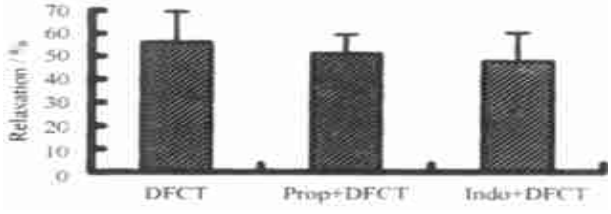
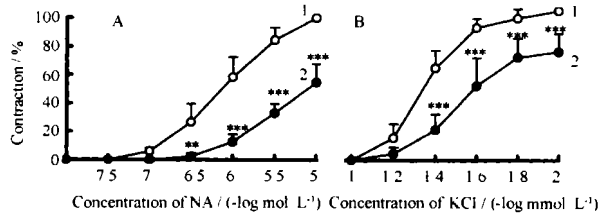


Fig. 2 Effects of Indo and Prop on relaxation induced by DFCT (20 mg/mL) in NA (10^{-6} mol/L) precontracted isolated rabbit aortic strips

2.3 Effects of DFCT on NA and KCl concentration-dependent contractile responses. The NA (Fig. 3 A) and KCl (Fig. 3 B) concentration-dependent contraction curves were shifted to the right after incubation with DFCT (40 mg/mL) in rabbit arterial strips. Maximal contractions for NA and KCl were reduced to (54.72 ± 4.58)% and (72.88 ± 4.28)% respectively ($P < 0.001$, $n = 7$). The PD_2 values in control and after incubation with DFCT (40 mg/mL) were (6.06 ± 0.09) and (5.07 ± 0.08) for NA, and were (1.71 ± 0.33) and (1.35 ± 0.20) for KCl, respectively.



1-control group; 2-DFCT 40 mg/mL

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$ *vs* control group

Fig. 3 Effects of DFCT (40 mg/mL) on NA (A) and KCl (B) concentration-dependent contraction curves in isolated rabbit aorta

3 Discussion

DFCT contains varied effective constituents, such as carthamone, carthamin, neocarthamin, safflower-yellow, 15α, 20β-dihydroxy-Δ^4-pregnen-3-one and so on[12], and its different preparations exhibit extensive pharmacological actions which were mainly related to cardiovascular system[8]. Some researchers reported that the decoction and water extract of DFCT and safflower-yellow could directly affect the tension of vascular smooth muscle, but the mechanism involved in its actions were not elucidated.

Results shown that the water decoction of DFCT could relax aortic strips precontracted with NA in the same manner as Ach, but did not affect their resting tension. It is now recognized that vasodilatory response to Ach are mainly mediated by release of nitric oxide (NO) from endothelium[13] and NO can stimulate soluble guanylyl cyclase in smooth muscle cells to increase the second messenger guanosine 3′, 5′-cyclic monophosphate (cGMP), which is thought to lead to relaxation largely via voltage-independent mechanism. In our experiments, denuded or incubated rabbit aortic strips with *L*-NNA, an inhibitor of NO synthesis significantly decreased the relaxation caused by DFCT. MB, an inhibitor of cGMP synthesis also could reduce the relaxation induced by the preparation. Our results suggest that *in vitro* relaxation of rabbit aorta caused by acute administration DFCT be dependent on endothelium and probably relate to cGMP.

The endothelium might also release a number of prostaglandins, either vasodilator or constrictor. In our experiments, Indo did not affect relaxation induced by DFCT in endothelium-intact aorta. Prop, an antagonist of adrenergic β receptor, also did not affect the vasorelaxation. These results indicate that the release of vasodilator prostanoids and adrenergic β receptor are not associated with aortic relaxation induced by DFCT.

Noradrenaline (NA) can activate receptor-operated calcium channels (ROCs) in cellular membrane of vascular smooth muscle and increase calcium influx, meanwhile can also activate G proteins and phospholipase C to produce inositol trisphosphate (IP_3) which causes calcium release from endoplasmic reticulum[14]. Potential dependent calcium channels (PDCs) are activated by depolarization of the plasma

membrane when the extracellular K^+ concentraction is increased[14]. In the present study, DFCT shifted the NA and KCl concentration-response curves to the right. These results might reflect the inhibition of calcium influx into the cellular cytoplasm which are supported by those previously reports that DFCT could markedly block calcium influx through ROCs and PDCs in cellular membrane of vascular smooth muscle according to ^{45}Ca across-membrane measurement[15]. In this way, the vasodilator effect of DFCT has been associated with an inhibitory modulation of calcium entry into vascular smooth muscle.

In conclusion, our results indicate that, in rabbit thoracic aorta, the water decoction of DFCT exhibits a vasodilatory effect on contraction induced by NA, which is related to endothelium and involved in NO. Also, this vascular relaxation probably is mediated by inhibition of calcium influx via ROCs and PDCs.

References:

[1] Wang H L. The anticoagulant effects of safflower yellow from safflower [J]. *Sci Tech Chin Tradit Med*, 1998, 5: 333-334.

[2] Huang Z L, Gao Q M, Cui Z M. Pharmacological studies on safflower yellow from safflower [J]. *Chin Tradit Herb Drugs* (中草药), 1984, 15: 12-14.

[3] Kasahars Y, Kumaki K, Katagiri S, *et al*. *Flos Carthami* extract and its component, stigmasterol, inhibit tumor promotion in mouse skin two-stage carcinogenesis [J]. *Phytother Res*, 1994, 8: 327-331.

[4] Huang Z L, Cui Z M, Ren Y, *et al*. The effect of safflower yellow from safflower on the endurance of anoxia in animals [J]. *Chin Tradit Herb Drugs* (中草药), 1985, 16: 27-29.

[5] Zhang H L, Nagatsu A, Watanabe T, *et al*. Antioxidative compounds isolated from safflower oil cake [J]. *Chem Pharm Bull*, 1997, 45: 1910-1914.

[6] Siow Y L, Choy P C, Leng W M. Effects of *Flors Carthami* on stress-activated protein kinase activity in the isolated reperfused rat heart [J]. *Mol Cell Biochem*, 2000, 207: 41-47.

[7] Shi M Y, Chang L F, He G B. Research on the stimulating aetion of *Carthamus tinctorius* L., *Angelica sinensis* (Oliv.) Diel and *Leonurus sibiricus* L. on uterus [J]. *China J Chin Mater Med* (中国中药杂志), 1995, 20: 173-175.

[8] Zheng H Z, Dong Z H, Yu Q. *Modern Research and Application of Traditional Chinese Medicine* (中药现代研究与应用) [M]. Beijing: Xueyuan Press, 1999: 2063-2089.

[9] Chang H T, Han H X, Tu P F, *et al*. Chemical compositions and pharmacological effects of safflower [J]. *World Phytomedicine*, 1999, 14(5): 201-203.

[10] Rong H Z. Studies on chemical compositions and pharmacological actions of safflower [J]. *J Jiangxi Coll Tradit Chin Med* (江西中医学院学报), 1997, 9: 45-46.

[11] Li H F, Li W, Zheng T Z, *et al*. A study of the mechanisms in relaxation induced by 17 β-estradiol in the isolated rabbit aorta [J]. *Arch Gynecol*, 2002, 266 (2): 101-104.

[12] Nobakht M, Fattahi M, Hoormand M, *et al*. A study on the teratogenic and cytotoxic effects of safflower extract [J]. *J Ethnopharmacol*, 2000, 73: 453-459.

[13] Plane F, Wiley K E, Jeremy J Y, *et al*. Evidence that different mechanisms underlie smooth muscle relaxation to nitric oxide and nitric oxide donors in the rabbit isolated carotid artery [J]. *Br J Pharmacol*, 1998, 123: 1351-1358.

[14] Karaki H, Ozaki H, Hori M, *et al*. Calcium movements, distribution, and function in smooth muscle [J]. *Pharmacol Rev*, 1997, 49: 157-230.

[15] Mo S W, Cheng H L, Liu N, *et al*. Influence of safflower on Ca^{2+} transmembrane influx in rat aorta [J]. *Chin Tradit Herb Drugs* (中草药), 1995, 26: 532-534.

人参茎叶皂苷对失血性休克大鼠糖皮质激素受体的影响

凌昌全[1], 李 敏[2], 苏永华[1], 李 勇[1], 黄雪强[1], 沈志雷[2], 谭金兴[3]*

(1. 第二军医大学长海医院 中医科, 上海 200433; 2. 第二军医大学 军队卫生学教研室, 上海 200433; 3. 第二军医大学 病理生理学教研室, 上海 200433)

摘 要: 目的 观察人参茎叶皂苷 (ginsenosides, GSS) 对失血性休克大鼠糖皮质激素受体 (GR) 的影响, 并分析其作用机制, 为研制及时抢救失血性休克患者的天然药物制剂提供实验依据。方法 雄性 SD 大鼠随机分为失血性休克组和对照组, 失血性休克组分别每日 ig 200, 100, 50 mg/kg GSS 水溶液, 对照组和模型组 ig 蒸馏水 2 mL, 共 10 d。以 [^{3}H] 地塞米松为配体, 用一点分析法测脑和肝胞液 GR 结合活性 (Rs)、半定量 RT-PCR 方法测肝胞液 GR mRNA 水平、放免法测血浆促肾上腺皮质激素 (ACTH) 和皮质酮 (GC) 浓度。结果 GSS 组大鼠脑和肝胞液的 GR 结合活性高于单纯失血性休克组, 其中以中剂量组最明显 ($P < 0.01$); GSS 组大鼠肝胞液 GR mRNA 表达水平高于单纯失血性休克组; GSS 组大鼠血浆 ACTH 和 GC 浓度和单纯失血性休克组没有明显差别。结论 GSS

* 收稿日期: 2002-08-24
基金项目: 国家杰出青年基金资助项目 (30025045); 国家自然科学基金面上项目 (30271675)

作者简介: 凌昌全 (1957—), 男, 安徽怀宁人, 医学博士, 博士研究生导师, 主任医师, 教授, 专业研究方向为中西医结合。