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Author(s)	Jin, HF; Shan, QX; Jiang, HD; Tu, J; Bruce, IC; Xia, Q
Citation	The 25th Annual International Conference of the IEEE Engineering in Medicine and Biology Society Proceedings, Cancun, Mexico, 17-21 September 2003, v. 1, p. 305-307
Issued Date	2003
URL	http://hdl.handle.net/10722/46970
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Proceedings of the 25th Annual International Conference of the IEEE EMBS
Cancun, Mexico • September 17-21, 2003

Vasorelaxant Effect of Total Flavones from Dendranthema Morifolium on Rat Thoracic Aorta

H-F. Jin[1], Q-X. Shan[1], H-D. Jiang[2], J. Tu[1], I.C. Bruce[3], Q. Xia[1]

[1]School of Medicine, [2]College of Pharmaceutical Science, Zhejiang University, Hangzhou; [3]Faculty of Medicine, The University of Hong Kong, Hong Kong, China
E-mail: xiaqiang@zju.edu.cn

Abstract—**To investigate the vasorelaxant effect of total flavones from Dendranthema morifolium (Ramat.) Tzvel. cv. Hangju (FDM), tension was recorded from rat thoracic aortic rings. FDM completely relaxed, in a dose-dependent manner, the contractions induced by either phenylephrine (PE) or a high concentration of KCl (60 mM) in rings with intact endothelium. Mechanical removal of the endothelium did not significantly modify the vasorelaxant effect of FDM. In endothelium-denuded aortic rings depolarized by 60 mM KCl, FDM inhibited Ca^{2+}-induced contraction. FDM also reduced the transient contraction elicited by PE in Ca^{2+}-free medium, but had no effect on active phorbol ester-induced contraction. Pretreatment of endothelium-denuded aorta with propranolol, a beta-adrenoceptor antagonist, significantly attenuated the relaxant effect of FDM. These results indicate that FDM induces an endothelium-independent relaxation in rat aortic rings. The mechanisms may include the activation of beta-adrenergic receptors, reduction in Ca^{2+} influx through the voltage-dependent and receptor-operated channels, and inhibition of intracellular Ca^{2+} release in vascular smooth muscle cells.**

Keywords—**Dendranthema morifolium, total flavones, aorta, beta-adrenoceptor**

I. Introduction

Dendranthema morifolium (Ramat.) Tzvel. cv. Hangju (DMH), belonging to the large chrysanthemum family, is widely used in China, especially in the south, either as a fragrant floral tea or as a cooling herb in traditional Chinese medicine. Components of DMH that have been identified include volatile oils, glycosides, adenine, amino acids, vitamins A and B, flavonoids and polyphenols [1,2]. We have shown that DMH increases contractility and coronary flow in perfused rat heart, and therefore protects against the reduction in contractility and coronary flow induced by ischemia/reperfusion [3,4]. However, the possible pharmacological effects of DMH on blood vessels have not been elucidated. We therefore hypothesized that total flavones purified from the aqueous extract of DMH might exert a direct vasorelaxant effect, contributing to the increase in coronary flow seen in the isolated perfused heart. After confirming the hypothesis, we investigated the mechanisms underlying the flavone-induced vasorelaxation.

II. Methodology

Total flavones from DMH

Dried flowers of chrysanthemum (Dendranthema morifolium (Ramat.) Tzvel. cv. Hangju) were obtained from Tongxiang City, Zhejiang Province. Total flavones (FDM) were extracted by Prof H.D. Jiang of the College of Pharmaceutical Sciences, Zhejiang University.

Preparation of rat aortic rings

Male Sprague-Dawley rats (240-300g) were killed by stunning followed by cervical dislocation. The thoracic aorta was rapidly removed, cleaned of fat and connective tissue, and cut into 3 mm rings. The rings were mounted in organ chambers containing 10 ml Kreb's solution at 37°C, bubbled with 95% O_2 and 5% CO_2 [5]. The rings were equilibrated for 1 h and the Kreb's solution was changed every 15 min. Changes in isometric tension were recorded by force transducers connected to a data acquisition system (MacLab, ADInstruments). Before each experiment, rings were stimulated with 60 mM KCl at least 3 times until a reproducible contractile response was obtained. The presence of functional endothelium was verified by the ability of acetylcholine (10^{-5} M) to induce more than 70% relaxation in rings precontracted with phenylephrine (PE, 10^{-6} M).

Experimental protocols

In the first series of experiments, after PE (10^{-6} M) or KCl (60 mM) elicited a steady contraction of rat aortic rings with or without endothelium, FDM was added cumulatively to induce a concentration-dependent relaxation. In experiments examining the involvement of beta-adrenergic receptors, rings without functional endothelium were incubated with propranolol (10^{-6} M), a beta-adrenoceptor antagonist, for 30 min before PE application. Tension was expressed as a percentage of PE- or KCl-induced contraction.

In the second series of experiments, aortic rings were incubated in Ca^{2+}-free solution containing 50 μM EGTA and 60 mM KCl for 20 min. Ca^{2+} was then added cumulatively to obtain a concentration-response curve. FDM at 5×10^{-2} $g\cdot L^{-1}$ was added 10 min before the addition of $CaCl_2$.

In the third series of experiments, the rings were exposed to Ca^{2+}-free solution with 50 μM EGTA for 15 min before the application of 10^{-6} M PE to induce the first transient contraction. The rings were then washed with

normal Krebs solution 3 times and incubated for at least 40 min to allow refilling of the intracellular Ca^{2+} stores. Subsequently, the medium was rapidly replaced with Ca^{2+}-free solution and the rings were incubated for 15 min. The second contraction was then induced by PE in the absence and presence of 5×10^{-2} g·L^{-1} FDM, which was added 10 min before PE application. In addition, after 10^{-6} M phorbol 12,13-diacetate, a protein kinase activator, induced a steady contraction in Ca^{2+}-free solution containing 50 μM EGTA, the effect of FDM was examined.

Data analysis and statistics

Data were expressed as mean ± S.E.M. Comparisons were made by the unpaired Student's t-test between two groups. Comparisons between multiple groups were made by one-way analysis of variance. $p<0.05$ was considered significant.

III. RESULTS

A Vasorelaxant effect of FDM

After 10^{-6} M PE induced a steady contraction, FDM was added cumulatively and induced a concentration-dependent relaxation (Fig. 1). FDM caused complete relaxation of endothelium-intact and -denuded aortic rings. Functional removal of the endothelium did not affect the FDM-induced relaxation (Fig. 1A). Similarly, FDM produced a concentration-dependent reduction of high K^+ (60 mM) induced contraction both in endothelium-intact and in endothelium-denuded arteries (Fig. 1B).

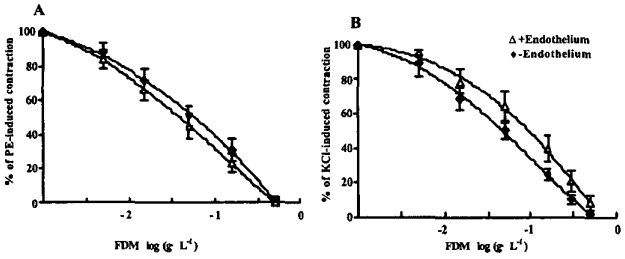

Fig. 1 Concentration-dependent effect of FDM on the PE (10^{-6} M) (A) or KCl (60mM) (B) induced contraction of endothelium-intact or endothelium-denuded aortic rings. Results are expressed as mean ± S.E.M. n=8-10.

B Effect of FDM on Ca^{2+}-induced contraction

Ca^{2+} induced a concentration-dependent contraction of rat aortic rings without endothelium depolarized by 60 mM KCl. Incubation of the aortic rings with 5×10^{-2} g·L^{-1} FDM significantly inhibited the Ca^{2+}-induced contraction (Fig. 2).

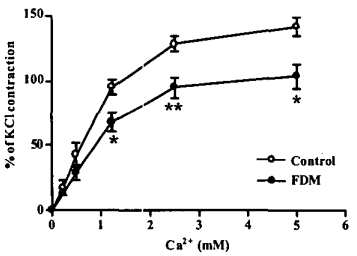

Fig. 2 Effect of FDM (5×10^{-2} g·L^{-1}) on the Ca^{2+}-induced contraction of rat aortic rings without endothelium depolarized with high-K^+. Results are expressed as mean ± S.E.M. of 5 observations. * $p<0.05$, ** $p<0.01$, compared with control.

C Effect of FDM on PE-induced contraction in Ca^{2+}-free solution

In endothelium-denuded rings in Ca^{2+}-free solution, a transient contractile response was elicited by 10^{-6} M PE. A second contraction was then induced by PE in the absence or presence of 5×10^{-2} g·L^{-1} FDM. Pretreatment of the aortic rings with 5×10^{-2} g·L^{-1} FDM for 10 min significantly reduced the ratio of the second contraction to the first contraction (Fig. 3).

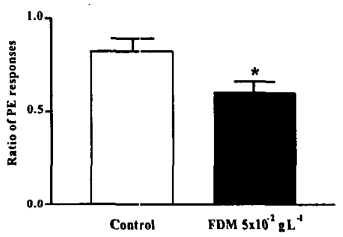

Fig. 3 Effect of FDM on the ratio of the contractile responses to PE of rat aortic rings without endothelium in Ca^{2+}-free solution. Results are expressed as mean ± S.E.M. of n observations (n=7 for control; n=9 for FDM group). * $p<0.05$, compared with control.

D Effect of FDM on phorbol ester-induced contraction in Ca^{2+}-free solution

We used phorbol 12, 13-di-acetate, a protein kinase C (PKC) activator, to evoke a sustained contraction of endothelium-denuded rings in Ca^{2+}-free solution with 50 μM EGTA. FDM at 5×10^{-1} g·L^{-1} did not affect the phorbol ester-induced contraction (Fig. 4).

E Effect of propranolol on FDM-induced relaxation in endothelium-denuded aortic rings

An attempt was made to test the possible involvement of beta-adrenergic receptors in the vasorelaxant effect of FDM. Pretreatment of the endothelium-denuded rings with 10^{-6} M propranolol, a beta-adrenoceptor antagonist, attenuated the relaxation induced by FDM without affecting the maximum relaxation (Fig. 5).

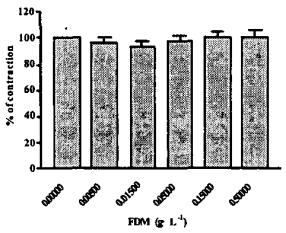

Fig. 4 FDM had no effect on the contraction induced by phorbol ester (10^{-6} M) in endothelium-denuded aorta in Ca^{2+}-free solution. Results are expressed as mean ± S.E.M. of 4 independent experiments.

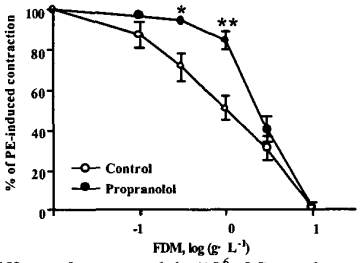

Fig. 5 Effect of propranolol (10^{-6} M), a beta-adrenoceptor antagonist, on FDM-induced vasorelaxation in endothelium-denuded rat aortic rings precontracted with phenylephrine (PE). Results are expressed as mean ± S.E.M. of n observations (n=10 for control; n=7 for propranolol). * $p<0.05$; ** $P<0.01$, compared with control.

IV. DISCUSSION

Although DMH is widely consumed in the south of China, to our knowledge, the potential vasorelaxant effect of FDM in rat aorta and its underlying mechanism are reported here for the first time.

FDM had a potent vasorelaxant effect; it completely relaxed the contractions induced by either PE or a high concentration of KCl. Also, FDM-induced vasorelaxation was observed in rat aorta with or without functional endothelium, suggesting that this effect is endothelium-independent. The release of endogenous vasodilators from endothelial cells, such as nitric oxide and prostacyclin, is not involved.

It is well known that KCl-induced contraction mainly results from the influx of Ca^{2+} upon depolarization of the cell membrane, which activates voltage-dependent L-type Ca^{2+} channels [6]. FDM inhibited KCl-induced vasoconstriction in endothelium-denuded rat aorta. Also, it significantly reduced the Ca^{2+}-induced contraction in aortic rings depolarized by KCl. These results demonstrate that FDM exerts its vasorelaxant effect, at least in part, by blocking the L-type Ca^{2+} channel. In addition, FDM significantly inhibited PE-induced contraction, suggesting that it might attenuate Ca^{2+} influx through receptor-operated Ca^{2+} channels as well.

PE is an α-adrenoceptor agonist. It causes vasoconstriction by opening receptor-operated Ca^{2+} channels, and more importantly by activating phospholipase C, leading to the formation of diacyl glycerol (DG), which activates myosin light chain kinase through PKC and IP_3, which causes IP_3-induced Ca^{2+} release from the sarcoplasmic reticulum [7,8]. Therefore, we further tested the hypothesis that the vasorelaxant effect of FDM might also be due to inhibition of intracellular Ca^{2+} mobilization from the sarcoplasmic reticulum and DG-PKC-activated myosin light chain kinase in vascular smooth muscle. The experiments were thus carried out in a calcium-free medium. Under this condition, PE-induced transient contraction was mainly due to IP_3-induced Ca^{2+} release from the sarcoplasmic reticulum. Our results showed that FDM inhibited the contractile response induced by PE in the absence of extracellular Ca^{2+} in endothelium-denuded aorta, suggesting a possible inhibitory effect of FDM on intracellular Ca^{2+} release. However, at a concentration range that relaxed PE- or KCl-precontracted aorta, FDM did not affect the phorbol ester-induced contraction, indicating that FDM had no effect on the PKC-mediated contractile mechanism in rat aortic rings.

Pretreatment of the endothelium-denuded rings with propranolol, a beta-adrenoceptor antagonist, attenuated the DMH-induced relaxation. This result suggests that activation of the beta-adrenoceptor mediates the vasorelaxant effect of FDM.

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