TFCM Induced Vasodilation of Isolated Rat Thoracic Aorta

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***Abstract*—By the way of isolated vessel perfusion, the direct effect of TFCM on blood vessel contraction was observed and its influence on blood vessel contraction caused by phenylephrine (PE) or potassium chloride, intracellular calcium releasing and extracellular calcium inflowing. TFCM did not directly relax or contract vascular rings, but TFCM could significantly inhibit the contraction of the vascular rings caused by phenylephrine (PE), and potassium chloride (compare with those of control, *P*>0.05), and the same inhibition to the contraction caused by intracellular calcium releasing and extracellular calcium inflowing (compare with those of control, *P*<0.05). TFCM could inhibit the contraction of vascular rings caused by phenylephrine and potassium chloride, whose possible mechanism was to inhibit intracellular calcium releasing and extracellular calcium inflowing.**

Keywords- total flavones from Chrysanthemum Morifolium Ramat. cv. Hangju (TFCM); aorta rings; vasodilation; calcium

I. INTRODUCTION

Chrysanthemum, a part of *Chrysanthemum Morifolium* Ramat. *cv*. Hangju's dry capitulum, were very abundant in Anhui, Zhejiang, Henan and somewhere else, one of which in Zhejiang was called Hangju, and one of famous Eight Zhe's. With being rich of Chrysanthemin, purine, amino acid, flavonoid, kind of vitamin and microelement and some researches, the Chrysanthemum can be used to heal coronary artery heart disease and hypertension, in the body heart, have obvious expanding coronary artery, helping increasing the flow of coronary artery, oxygen consumption of cardiac muscle, slower the heart rate and affecting myocardial contraction force [1,2]. Recent researchers have found that the flavonoid can help reduce blood press [2-5]. We were to observe the effects TFCM place on the thoracic aorta contraction of isolated rat, and make further study about its mechanisms.

II. MATERIAL AND METHODS

A. Reagents and Equipment

TFCM were from Pharmaceutical analysis laboratory Pharmaceutical Sciences College of Zhejiang University. Phenylephrine (PE), Acetycholine (Ach), N-nitro-*L*-arginine-methyl-ester (*L*-NAME), methylene blue, indomethacin (Indo) and EGTA were from Sigma (America), and the others were made in China. Krebs-Henseleit (K-H) (mmol/L) was made from NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, Glucose 5.5 and $CaCl_2$ 2.5. Male Sprague-Dawley rats (250 ± 10 g) were from Academy of Medical Sciences Animal Centre Zhejiang.

B. Establish and Stabilize the Thoracic Aortic Ring

We hit the rat and make a through bloodletting, pull out the aorta instantly, rid the fat and connective tissue around the aorta carefully, then cut a 3-4 mm long vascular circle. Hang the vascular circle in the beaker containing 5.0 ml K-H (37 ℃ ± 0.5 ℃ constant temperature pumping the mixed gas containing 95% O_2 and 5% CO_2), the changing of tension was recorded into the Medlab Biological signal collection and disposal system. Given 2.0 g initial tension and kept for 60 min, when the vascular circle was kept for 30 min in condition of 0 g, during which change the K-H every 15 min. Contract the vascular circle with stimulating by 120 mmol/L KCl, after that, wash the circle 3 times until it back to initial condition, repeat 3 times.

Use the Ach test the integrity of vessel endothelium. Change the liquid, when the vascular circle was stable, then pour into 10^{-5} mol/L PE to stimulate vascular circle after it reaches to the highest contraction limit, and pour into 10^{-5} mol/L Ach, meantime, test the diastole range. If the diastole range greater than 80%, it means that the vessel endothelium was integrated, also, the damage to the vessel endothelium was little, which means the integrity was good, called integrated endothelium group (E+); if there was no diastole or the diastole range was less than, it means that the vessel endothelium was damaged and the function was not completely integrated, which was called non-integrated endothelium group (E-). After checking the endothelium function and washing the vascular circle to the initial condition, waiting for about 30 min, during which time change the liquid every 15 min, and make a further test of the effects of medicine on the vascular circle.

With maximum shrinkage rate of 100% induced by PE, the proportion of vessel tension range within medicine of maximum shrinkage rate of 100% induced by PE reflect the change of vessel tension.

C. Group of Experiment

TFCM's influence to foundation tension of vascular circle group, vascular circle with integrated endothelium or without endothelium, take cumulative dosing method, pour into TFCM every 10min, and record the curve of change of vascular tension. TFCM's influence to PE's effects on tension of contracted vascular circle group, vascular circle with integrated endothelium or without endothelium, pour into PE and after the contraction of vascular circle was stable, pout into TFCM every 10 min, and record the curve of change of vascular tension. TFCM's influence to KCl's effects on tension of contracted vascular circle group, vascular circle with integrated endothelium or without endothelium, input KCl (20 mmol/L), and after the contraction of vascular circle was stable, pour into TFCM every 10 min, and record the curve of change of vascular tension. TFCM's influence to PE's effects on tension of contracted vascular circle group in the condition of non-calcium K-H, vascular circle without endothelium, with the moist of K-H after 40 min, changed into non-Ca^{2+} K-H （containing 5×10^{-5} mol/L EGTA）about 15 min, then pour into PE to make the vessel contracted, after the contraction was stable, mark the level of contraction *con1*, pour into K-H to the baseline, after 40 min, change into non-Ca^{2+} K-H for 15 min, then pour into TFCM (0.1 g/L) for 10min, then pour into PE to make the vessel contracted, after the contraction was stable, mark the level of contraction *con2*. Compare the two data, calculate *con1/con2*. Compare the value in the condition of whether the luteolin exists. TFCM's influence to PE's effects on tension of contracted vascular circle group in the condition of non-calcium K-H, vascular circle without endothelium，with the moist of K-H after 40 min, changed into non-Ca^{2+} K-H （containing 5×10^{-5} mol/L EGTA）,meantime, pouring into KCl (120 mmol/L), moist for 20min, then pour into $CaCl_2$ (2.5×10^{-4} mol/L and 5×10^{-3} mol/L) step by step, observe the change of vascular tension. In the TFCM group, before pouring into $CaCl_2$, pour into TFCM (0.1 g/L) 10 min ahead. L-NAME's influence to TFCM group, vascular circle with endothelium, use L-NAME (10^{-4} mol/L) to make moist for 10 min, then use PE (10^{-5} mol/L) to contract vessel, when reaching to the steady state, whether pouring into TFCM (0.1 g/L) or not, observe 10 min. Methylene blue's influence to TFCM group, vascular circle with endothelium, with methylene blue (10^{-5} mol/L) moist for 10 min. then use PE (10^{-5} mol/L) to contract the vessel, after reaching the steady state, whether pouring into TFCM (0.1 g/L), observe 10 min. Indo's influence to TFCM group, vascular circle with endothelium, with Indo (10^{-5} mol/L) moist for 10 min, then use PE (10^{-5} mol/L) to contract the vessel, after reaching the steady state, whether pouring into TFCM (0.1 g/L), observe 10 min.

D. Statistical Analysis

All the experimental data were expressed as Mean ± SD and analyzed by one-way or two-way ANOVA and independent sample t test from Excel 2003 database and the SPSS 11.5 statistical package. The probability value of 0.05 was accepted as significant for differences between groups of data.

III. Results

A. TFCM's Influence to Foundation Tension of Vascular Circle

The different concentration of 0.001, 0.003, 0.01, 0.03, 0.10, 0.30 (g/L) TFCM affect the vascular circle, there were no obvious change (no pouring into TFVM pouring into TFCM 100.1 ± 0.19 *vs* 99.99 ± 0.20), which indicate that it does no effects to the change of contraction or diastole (P>0.05).

B. The Influence Caused by PE

The inhibition to contraction of endothelium and non-endothelium vascular circle caused by 10^{-5} mol/L PE from 0.001, 0.003, 0.01, 0.03, 0.10, 0.30 (g/L) TFCM was obvious, the high concentration was, the strong the inhibition was, which present concentration-response relationship, the difference between the two group was obvious (P<0.01), the effect from TFCM between the endothelium and the non-endothelium was of great difference, as shown in Fig 1.

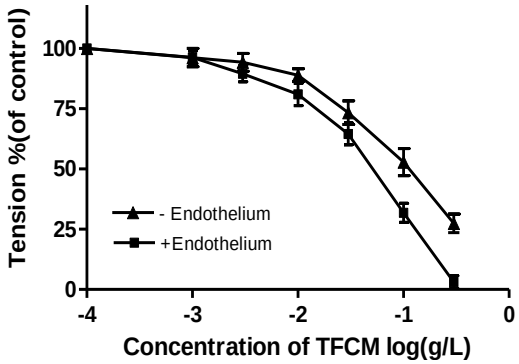

Fig. 1 Effect of total flavones from *Crysanthemum morifolium* Ramat. *cv.* Hangju (TFCM) on tension of the phenylephreine (PE) precontracted aorta rings of rat. Pooled data of mean normalized vessel tension obtained from PE precontracted aorta rings with or without endothelium.

C. The KCl' Influence to Contraction of Vascular Circle

The inhibition to contraction of endothelium vascular circle caused by 120 mmol/L KCl from 0.001, 0.003 (g/L) TFCM was obvious (P<0.01), the high concentration was, the strong the inhibition was, which present concentration-response relationship, the difference among the groups was obvious (P<0.01), the inhibition to non-endothelium was weak from different concentration of TFCM, when it comes to the low concentration, the inhibition disappear, the effect from TFCM between the endothelium and the non-endothelium was of great difference (P<0.01) , as shown in Fig 2. TFCM's influence from PE to the contraction in the non-calcium liquid being in the moist of non-calcium K-H (0.1 mmol/L EGTA) PE (10^{-5} mol/L) that can induce the brief contraction of vascular circle TFCM (0.1 g/L) (P<0.01) , as shown in Fig 3.

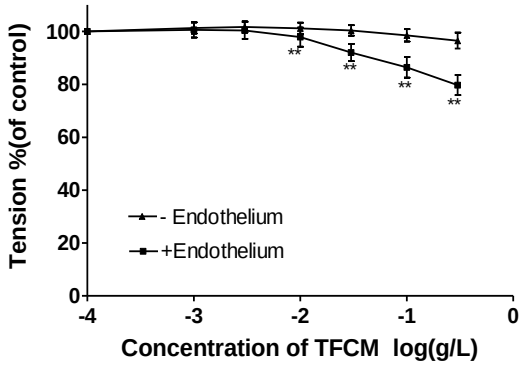

Fig.2 Effect of total flavones from Crysanthemum morifolium Ramat. cv. Hangju (TFCM) on tension of KCl (120 mmol/L) precontracted aorta rings of rat. Pooled data of mean normalized vessel tension obtained from KCl precontracted aorta rings with or without endothelium.Values were expressed as mean ± SD (n=9). ***P*<0.01.

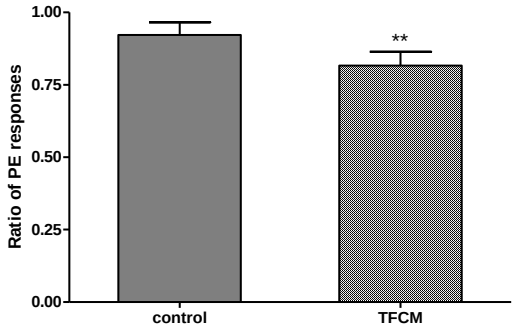

Fig 3 Effect of total flavones from Crysanthemum morifolium Ramat. cv. Hangju (TFCM) on the ratio of the contractile responses to PE (10^{-5} mol/L) of rat aortic rings without endothelium in Ca^{2+}–free solution. Values were expressed as mean ± SD (n=10). ***P*<0.01.

D. TFCM's Influence to $CaCl_2$ Dose-reponse Curve

In the non-calcium K-H, the higher the concentration of the $CaCl_2$ was, the vascular circle depolarized by (120 mmol/L) in advance contracts more. But if it was proceed by TFCM (0.1 g/L) ahead for 10 min, the curve moves right non parallel, and the maximum of the contraction decrease, as shown in Fig 4.

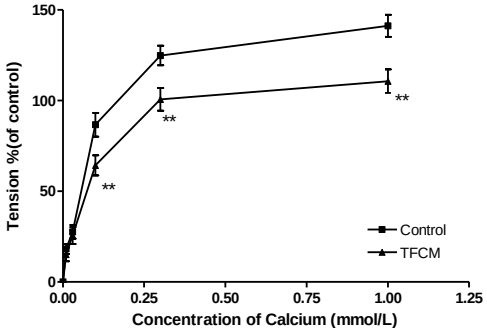

Fig.4 Effect of total flavones from Crysanthemum morifolium Ramat. cv. Hangju (TFCM) on the Ca^{2+}–induced contraction of rat aortic rings without endothrlium depolarized with high K^+. Results were expressed as mean ± SD (n=9). ***P*<0.01.

E. L-NAME and Methylene Blue's Influence to TFCM

Activate the GC by NO, which cause cGMP higher, then to make vessel diastole. In order to know whether NO and GC involve in TFCM's influence to the vessel diastole, the NO synthase inhibitors *L*-NAME and GC inhibitor methylene blue were used respectively. From the chart, we know that after the using of *L*-NAME (10^{-4} mol/L) and methylene blue (10^{-5} mol/L) to vascular circle, the PE's effect weakens if the TFCM (0.1 g/L) involve in the PE's influence to contraction of endothelium vascular circle. If we make moist of vascular circle with *L*-NAME (10^{-4} mol/L) and methylene blue (10^{-5} mol/L), no effect was put on the tension of vessel, as shown in Fig 5.

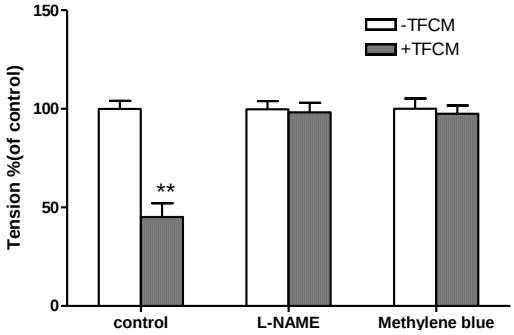

Fig. 5 Effect of pretreatment with (10^{-4} mol/L) or Methylene blue (10^{-5} mol/L) on the action of total flavones from Crysanthemum morifolium Ramat. cv. Hangju (TFCM, 0.1 g/L) in the endothelium-intact aorta rings. Values were expressed as mean ± SD (n=10 for L-NAME and n=9 for Methylene blue). ***P*<0.01.

F. Indo's Influence Involved with TFCM to Vessel

We want to know that if the cyclooxygenase involves in the influence of vessel diastole, and observe the effect that TFCM brings after the vascular circle with endothelium was processed by epoxy synthase inhibitor Indo, after that, the effect that TFCM brings on the PE's influence of diastole vascular circle with endothelium weakens. But if it was processed by Indo (10^{-5} mol/L) alone, there was no obvious effect placing on PE's influence to vessel tension (*P*>0.05), as shown in Fig 6.

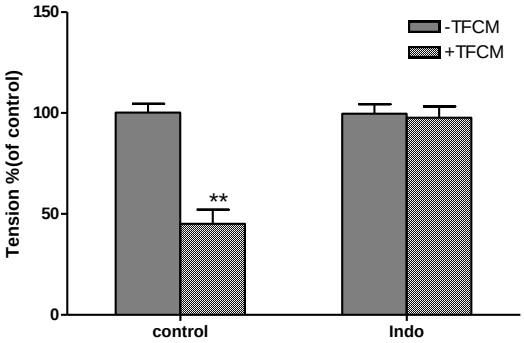

Fig. 6 Effect of pretreatment with indomethacin (Indo, 10^{-5} mol/L) on the action of total flavones from Crysanthemum morifolium Ramat. cv. Hangju (TFCM, 0.1 g/L) in the endothelium-intact aorta rings. Values were expressed as mean ± SD (n=10). ***P*<0.01.

IV. CONCLUSIONS

We found that though TFCM affects the vascular circle not so obviously, it has an obvious inhibition on PE and KCl's influence to the contraction of vascular circle, which present a Dose-effect relationship, and has endothelium-dependent dilation.

PE's selective exited α_1- receptor, made the phosphatidylinositol hydrolyzed, and generates 1, 3, 5-three phosphoinositide (IP3) and 1, 2-diester acyl glycerin (DG), which caused sarcoplasmic reticulum intracellular calcium to release, meantime, promote receptors in the cell membrane open the calcium channel to let the extracellular calcium flow internal, which leads to increased intracellular calcium, and the smooth muscle contract. TFCM can make the Aortic smooth muscle of rat diastole, also, the dose of the inhibition contract depends on the intracellular calcium and extracellular calcium, and it maybe lower calcium by restraining the releasing intracellular calcium and the into-flowing of extracellular calcium, which adjusts the smooth muscle tension. KCl (120 mmol/L) can depolarize the cell membrane, and open the VDC, causing the extracellular calcium flow into, and the calcium concentration was higher, and the smooth muscle contracts. The dose of TFCM leading to the independent diastole of KCl's leading to the contraction of vessel smooth muscle, but the range was small. Pour PE into the liquid after pouring into non calcium K-H with EGTA, at which time, the contraction of vascular circle was caused by the way of phosphoinositide, which acts on endoplasmic reticulum, making the calcium releasing stored in phosphoinositide. We found that, under this circumstance, TFCM can restrain the contraction caused by PE, which indicates that it can inhibit the releasing of intracellular calcium. With the moist of non calcium K-H mixed with high concentration K^+, at which time, the cell membrane was in the state of depolarization, and Voltage dependence of calcium channel was in active state, then pouring Ca^{2+}, at which time, the contraction of vascular was caused by the process of extracellular calcium's flowing into cell.

Endothelial cells acted on the tension of adjusting vessel smooth muscle by releasing matter of diastole [5-8]. NO was considered as the most endothelium-derived relaxing factor that release from the endothelial cells [7, 9-13] whose mechanism has been known. NO activity the soluble guanylyl cyclase (sGC), after it was compounded in the endothelial cells, causing the level of cyclic guanosine monophosphate (cGMP) increase, as a result of which, it make diastole of the vessel. The latter decreases the level of calcium influx by cGMP and cyclin-dependent kinases, and increases enzyme's absorbing of calcium or acting on contractile protein's dephosphorylation, as result, the vessel diastole [12-15]. In our research, NO Synthase inhibitors *L*-NAME can block TFCM's acting on diastole of vessel. And Guanylate Cyclase inhibitor methylene blue also can block TFCM's endothelium-dependent dilation of acting on vessel.

Based on the result, we infer that TFCM's effect of diastole of vessel can either place obvious inhibition on receptor dependence calcium channel, or do the same to voltage dependence calcium channel. But the separation work of TFCM's Effective molecular was still on, so its mechanism needs further study.

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REFERENCES

[1] Hajimehdipoor H, Kondori BM, Amin GR, "Development of a validated HPLC method for the simultaneous determination of flavonoids in Cuscuta chinensis Lam by ultra-violet detection," Daru, vol. 20, pp. 57, 2012.

[2] HD Jiang, Q Xia, WH Xu, "Chrysanthemum cardiovascular pharmacological effects and its mechanism research progress," Shijie Kexue Jishu Zhongyao Xiandaihua, vol. 4, pp. 31-4, 2002.

[3] Wang DQ, Zheng XX, Yin ZY, "Activating effect of citrus flavonoids on neuromedin U2 receptor and analysis on siRNA interference," Zhongguo Zhong Yao Za Zhi, vol. 37, pp. 3462-6, 2012.

[4] Ajay M, Gilani AU, Mustafa MR, "Effects of flavonoids on vascular smooth muscle of the isolated rat thoracic aorta," Life Sci, vol. 74, pp. 603-12, 2003.

[5] Herrera MD, Zarzuelo A, Jimenez J, "Effects of flavonoids on rat aortic smooth muscle contractility: structure-activity relationships," Gen Pharmacol, vol. 27, pp. 273-7, 1996.

[6] Chen H, Li L, Zhang Y, "Separation of purines, pyrimidines, pterins and flavonoids on magnolol-bonded silica gel stationary phase by high performance liquid chromatography," Se Pu, vol. 30, pp. 1062-7, 2012.

[7] Askari G, Ghiasvand R, Paknahad Z, "The effects of quercetin supplementation on body composition, exercise performance and muscle damage indices in athletes," Int J Prev Med, vol. 4, pp. 21-6, 2013.

[8] Rembold CM, "Regulation of contraction and relaxation in arterial smooth muscle," Hypertension, vol. 20, pp. 129-37, 1992.

[9] Moncada S, Palmer RM, Higgs EA, "Nitric oxide: physiology, pathophysiology, and pharmacology," Pharmacol Rev, vol. 43, pp. 109-42, 1991.

[10] Furchgou RF, Vanhoutte PM, "Endothelium-derived relaxing and contracting factor," FASEB J, vol. 3, pp. 2007-18, 1989.

[11] Palmer RM, Ferrige AG, Moncada S, "Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor," Nature, vol. 327, pp. 524-6, 1987.

[12] Rapoport RM, Mured F, "Agonist-induced endothelium-dependent relaxation in rat thoracic aorta may be mediated through cGMP," Circ Res, vol. 52, pp. 352-7, 1983.

[13] Vaandrager AB, de Jonge HR, "Signalling by cGMP-dependent protein kinases," Mol Cell Biochem, vol. 157, pp.23-30, 1996.

[14] Wu T, McCallum JL, Wang S, "Evaluation of antioxidant activities and chemical characterisation of staghorn sumac fruit (Rhus hirta L.)," Food Chem, vol. 138, pp. 1333-40, 2013.

[15] Czaplińska M, Czepas J, Gwoździński K, "Structure, antioxidative and anticancer properties of flavonoids," Postepy Biochem, vol. 58, pp. 235-44, 2012.