Antihypertensive Effect of Total Flavonoid Fraction of *Astragalus complanatus* in Hypertensive Rats

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Abstract

The purpose of the present study was to quantify the antihypertensive effect of the total flavonoid (TF), extracted from the seed of *Astragalus complanatus* R. Brown , and to observe its effect on the renin-angiotensin system (RAS) in both renal hypertensive rats (RHR) and spontaneously hypertensive rats (SHR). RHR were created by the two-kidney one clip (2K1C) method. Systolic blood pressure was measured in conscious rats by the tail-cuff method. Plasma angiotensin II (AngII) and plasma renin activity (PRA) were measured with radioimmunoassay at 60 min after drug administration. The effects of TF on cardiac hemodynamics were also recorded in anesthetized RHR and SHR. TF was given by oral administration in low dose (100 mg/kg) and high dose (200 mg/kg) respectively. Compared to pre-administration control, TF induced an obvious decrease in systolic blood pressure in conscious normotensive Wistar rat, RHR and SHR. In the three groups the systolic blood pressure reached the lowest value at 60 min after TF. TF also induced a significant decrease in blood pressure in anesthetized RHR and SHR. At 60 min after treatment of TF, mean arterial pressure in high dose group (200 mg/kg) was decreased by 17% in RHR and by 17% in SHR respectively ($P < 0.01$). The depressor effect of TF lasted for at least 60 min. Cardiac output, heart rate and ±dp/dtmax did not change. Conversely, total peripheral resistance was significantly decreased. The decrease in plasma AngII was found in both RHR and SHR. On the contrary, PRA increased at the same time. These findings suggested that TF is effective in reducing blood pressure in both RHR and SHR. The antihypertensive action of TF was attributed to a decrease in TPR secondary to a decrease in plasma concentration of AngII caused by TF.

Key Words: ***Astragalus complanatus*, total flavonoid, hypertension, antihypertensive effect, hemodynamics**

Introduction

Astragalus complanatus R. Brown is a widely used herbal material in traditional Chinese medicine. According to China pharmacopeia, its seeds can act primarily on kidney and liver channels, and are often used for antiaging therapy and improving the function of sexual performance (13). During the past two decades, a great deal of research was done to study its chemical constituents and pharmacological effects. Total flavonoid (TF) is an active fraction extracted from its seed. Previous studies performed in animals have demonstrated the antihypertensive efficacy of TF in anesthetized, normotensive dogs and rats (16, 17). However, so far it is unclear whether TF lowers blood pressure in hypertensive animals, such as in renovascular hypertensive rat (RHR) or in the spontaneously hypertensive rat (SHR). The present study was designed to determine whether TF is effective in reducing blood pressure in RHR or in

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SHR, and to explore the underlying mechanism.

Materials and Methods

Animals

Adult spontaneously hypertensive male rats (SHR, Wistar strain) were purchased from Cardiovascular Hospital of Beijing. Adult male Wistar normotensive rats were purchased from the animal breeding center of Shandong University. The experiments were approved by Shandong University Animal Care and Use Committee. A two-kidney one-clip (2K1C) model of RHR was induced in adult male Wistar rats , weighing 150 to 180 g as previously described by Mai *et al*. (8). Briefly, a silver clip with 0.2 mm internal diameter was placed around the left renal artery through a flank incision of the rat under pentobarbital sodium anesthesia (40 mg/kg). The right kidney remained untouched. Sham-operated rats underwent a similar procedure with manipulation of the left renal artery, without permanent application of a clip.

Systolic Blood Pressure Recording

Systolic blood pressure was measured by a tail-cuff method described by Bunag *et al*. (1) in conscious normotensive Wistar rat, RHR and SHR. The rats were divided into three groups respectively, namely, normal saline group (NS), low dose group (TF100, 100mg TF/Kg) and high dose group (TF200, 200 mg TF/Kg). The rat was enclosed in a warmed chamber (30°C). Then, a cuff is placed around the tail and inflated above the systolic pressure. This caused pulsations at a more distal pulse sensor (Mode HX-II, Hunan Medical University) to cease. As the cuff was slowly deflated, the reappearance of pulsations was observed, and the cuff pressure was taken to be the systolic pressure in the tail artery. Tail cuff pressure was obtained by 3 successive readings in average. Throughout the study, systolic blood pressure was measured before and at 15, 30, 45, 60, 90 and at 120 min after treatment of TF.

Hemodynamic Recording

Comprehensive hemodynamic recordings were made in RHR and SHR anesthetized with pentobarbital sodium (40 mg/kg). The rats were divided into three groups respectively, namely, normal saline group (NS), low dose group (TF100) and high dose group of TF (TF200). The rat was intubated and ventilated under anesthesia. Normal saline was administered through the femoral vein to compensate for perioperative fluid losses. A femoral artery was cannulated to monitor arterial pressure using a pressure transducer (model TP-400T, Nthon Kohden, Japan). After the heart was exposed *via* a midline sternotomy, the pericardium was incised, and a polyethylene catheter (PE50) connected to a pressure transducer (model TP-400T, Nthon Kohden, Japan) was inserted into the left ventricle. Its tip was positioned at the midventricular level, after which the catheter was secured in place with a purse-string suture. Systolic pressure (SP), diastolic pressure (DP), mean arterial pressure (MAP), left ventricular pressure (LVP), and dp/dt of LVP (with AP-621 amplifier and ED-601G differentiator) were recorded using a RM-6000 polygraph (Nthon Kohden, Japan). A probe of MF-1200 electromagnetic blood flowmeter was placed around the aortic root to measure cardiac output (CO). Limb lead ECG (lead II) was recorded to measure heart rate (HR). The total peripheral resistance (TPR) was calculated. All the hemodynamic parameters were measured before and at 15, 30, 45, 60, 90 and 120 min after treatment of TF.

Measurement of Plasma Angiotensin II (AngII) and Plasma Renin Activity (PRA)

Blood samples were obtained from the conscious normotensive Wistar rat, RHR and SHR at 60 min after TF administration. The rats were also divided into three groups respectively, namely, normal saline group (NS), low dose group (TF100) and high dose group (TF200). Plasma AngII and PRA were measured with radioimmunoassay as described by Shimamoto *et al*. (14).

Drugs

TF (purity 98.2%, quality control standard 90 ± 10%, No 010308) was supplied by Institute Basic Science of Medicine, Shandong Academy of Medical Sciences. The solvent of TF is distilled water. TF was given by oral administration at two doses, low dose (100 mg/kg) or high dose (200 mg/kg). Reagents for measurement of plasma AngII and PRA were purchased from Beijing North Institute of Biological Technology, Beijing, China.

Statistical Analysis

The data were expressed as mean ± S.D. Student's paired *t*-test or one way ANOVA test was used for statistical analysis. The criterion for significance was $P < 0.05$.

Results

Effect of TF on Systolic Blood Pressure in Conscious Normotensive Wistar Rats, RHR and SHR

As shown in Table 1, TF induced an obvious

Table 1. Effect of TF on systolic blood pressure (%) in conscious normotensive Wistar rats, RHR and SHR

		Systolic blood pressure (%)						
	n	0	15	30	45	60	90	120 min
Wistar-rat								
NS	8	100	101±2	101±3	99±2	98±3	99±3	98±3
TF100	8	100	102±3	99±2	97±2	94±2*	100±3	102±3
TF200	8	100	100±3	101±3	96±3	90±3*	95±3*	99±3
RHR								
NS	10	100	100±4	99±5	98±4	97±4	98±5	98±6
TF100	10	100	99±4	99±3	98±5	91±5**	92±6*	98±6
TF200	10	100	98±6	98±6	97±7	89±6**	91±5**	96±6
SHR								
NS	8	100	99±5	99±4	97±4	100±4	101±6	99±5
TF100	8	100	98±5	95±6	94±5*	91±7**	97±6	99±8
TF200	8	100	98±6	94±5*	89±8**	86±6**	95±7	98±5

Data are expressed as mean ± S.D.
*$P < 0.05$, **$P < 0.01$, as compared with pre-administration of drugs (0 min).

decrease in systolic blood pressure, which reached its lowest value at 60 min after TF. Systolic blood pressure in high dose group (TF200) decreased by 10% ($P < 0.01$) in normotensive Wistar rat, by 11% in RHR ($P < 0.01$) and by 14% in SHR ($P < 0.01$), respectively, and then went up to the pre-administration level in 120 min.

Effect of TF on Hemodynamics in Anesthetized RHR and SHR

TF induced a significant decrease in blood pressure in both RHR and SHR. The blood pressure reached its lowest value at 60 min. SP, DP, MAP decreased by 12%, 20%, and 17%, respectively ($P < 0.01$) in high dose group in RHR. The depressor effect of TF lasted for at least 60 min (Table 2). The blood pressure in SHR declined at 15 min after treatment of TF, which is earlier than that in RHR (Table 3). TPR significantly decreased in parallel with the change in blood pressure. However, CO, ±dp/dtmax and HR did not change significantly in both RHR and SHR.

Effect of TF on Plasma Ang II and PRA in Conscious Normotensive Wistar Rats, RHR and SHR

Table 4 summarizes the changes of the plasma AngII and PRA after treatment of TF. Compared with the control group, plasma AngII was found to decrease after treatment of TF in the three groups. The effect of high dose TF on plasma AngII was greater than that of the low dose in both RHR and SHR. On the contrary to plasma AngII, TF increased PRA in the three groups.

Discussion

As reviewed by Middleton, Jr. *et al*. (10), flavonoids are nearly ubiquitous in plants. Certain plants and spices containing flavonoids have been used for thousands of years in traditional Oriental medicine. Flavonoids are rich in seeds, citrus fruits, olive oil, tea, and red wine. They are low molecular weight compounds composed of a three-ring structure with various substitutions. The basic structure is comprised of two benzene rings (A and B) linked through a heterocyclic pyran or pyrone (with a double bond) ring (C) in the middle (Fig. 1). Flavonoids can be subdivided according to the presence of an oxy group at the position 4, a double bond between carbon atoms 2 and 3, or a hydroxyl group in position 3 of the C ring. In the flavonoid structure, a phenyl group is usually substituted at the 2-position of the pyrone ring. The substitution of isoflavonoids is at the 3-position.

The flavonoids have long been recognized to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities (9). In recent years, there has been a major rekindling of interest in the studies of plant flavonoids on cardiovascular system. Stern *et al*. (15) demonstrated that baicalein strikingly reduced the *in vitro* contractile response of artery rings to angiotensin II. In their study on isolated rat vascular smooth muscle, Duarte *et al*. (5) found that the contractile responses induced by high KCl, Ca^{2+} and PMA were inhibited by quercetin in a concentration-dependent manner. Study of the protective effect of silybin on spontaneously

Table 2. Effect of TF on hemodynamics in RHR

	0	15	30	45	60	90	120 (min)
SP (%)							
NS	100	100±3	99±3	99±5	98±4	97±3	98±5
TF100	100	99±3	97±3	94±7*	91±4**	95±4*	97±6
TF200	100	99±3	93±6**	89±6**	88±10**	94±4**	98±5
DP (%)							
NS	100	101±5	99±6	98±5	97±4	99±4	98±3
TF100	100	98±4	95±7	89±5**	84±6**	91±6*	99±7
TF200	100	98±8	88±8**	82±9**	80±8**	90±8*	99±8
MAP (%)							
NS	100	101±5	99±5	98±4	97±6	98±6	98±4
TF100	100	98±6	96±6*	91±7*	86±7**	92±6*	98±8
TF200	100	98±4	90±7**	85±5**	83±7**	91±5*	98±6
HR (beats/min)							
NS	295±10	300±13	298±13	294±14	292±11	293±9	297±13
TF100	300±12	308±12	295±14	293±14	290±13	301±13	299±12
TF200	298±11	295±10	299±13	295±8	292±12	293±7	295±6
LVP (mmHg)							
NS	176±13	180±10	179±12	174±9	170±11	173±8	175±11
TF100	180±12	179±10	179±13	177±12	175±9	177±10	177±12
TF200	178±10	171±12	168±8	168±11	169±7	171±9	174±13
+dp/dtmax (mmHg/s)							
NS	3890±200	3900±220	3750±250	3800±185	3740±240	3850±210	3825±235
TF100	4000±250	3950±230	3900±245	3920±229	3860±260	3940±205	3960±275
TF200	3800±300	3778±210	3780±235	3750±230	3739±240	3750±180	3740±220
-dp/dtmax (mmHg/s)							
NS	2800±210	2845±195	2680±220	2700±230	2690±235	2790±215	2915±190
TF100	3000±215	3000±225	3000±240	2950±210	2930±250	3100±260	3050±150
TF200	2850±223	2880±240	2800±258	2750±230	2750±230	2785±210	2900±200
CO (%)							
NS	100	99.2±5.4	100.5±5.0	99.7±4.8	98.3±6.0	98.5±7.5	98.3±6.6
TF100	100	99.8±6.6	99.4±4.5	101.4±7.6	101.2±6.5	100.8±9.1	99.4±7.0
TF200	100	101.6±10.2	98.9±8.9	98.2±8.0	98.8±6.8	100.4±8.1	102.4±9.8
TPR (%)							
NS	100	100.8±5.6	99.7±5.6	101.2±6.6	102.2±7.5	99.5±8.3	98.7±7.1
TF100	100	99.6±1.3	98.5±1.3	95.2±4.4*	92.4±4.2*	94.1±3.9	96.3±3.1
TF200	100	99.1±1.3	97.1±1.5	94.6±6.0*	89.7±5.9**	92.1±4.7*	98.7±3.7

Data are expressed as mean ± S.D., n = 10 in each group

*$P < 0.05$,**$P < 0.01$, compared with pre-administration of drugs (0 min)

hypertensive rats subjected to acute coronary artery occlusion performed by Chen *et al*. (2) indicates that silybin reduces blood pressure and the severity of ventricular hypertrophy. The effect of flavone on myocardial postischemic reperfusion recovery has been studied by Ning *et al*. (12). They found that flavone treatment caused significantly better recovery of left ventricular developed pressure and decline of end-diastolic pressures. However, so far the study of TF on cardiovascular system in hypertensive animals has not been reported.

In the present study, TF was used to investigate its hypotensive effect and the underlying mechanism in RHR and SHR. The results showed that TF reduced blood pressure in RHR and SHR. Its depressive effect was mainly attributed to a decrease of TPR which was paralleled to the decrease of plasma AngII. Furthermore, our results also showed that along with

Table 3. Effect of TF on hemodynamics in SHR

	0	15	30	45	60	90	120 (min)
SP (%)							
NS	100	99±5	98±8	98±7	97±6	97±8	98±5
TF100	100	98±7	95±5	90±5*	89±8*	94±7	99±6
TF200	100	93±5*	91±5**	88±5**	88±7**	93±4**	98±7
DP (%)							
NS	100	98±5	98±7	97±5	97±8	99±6	98±4
TF100	100	95±4	91±4*	85±4*	82±5**	93±6	98±4
TF200	100	89±5*	86±5*	80±4**	79±5**	89±4*	97±5
MAP (%)							
NS	100	98±7	98±6	97±7	97±6	98±6	98±4
TF100	100	96±4	92±5*	87±4*	86±5*	93±5*	98±5
TF200	100	91±4*	88±4**	83±3**	83±4**	91±4*	97±4
HR (beats/min)							
NS	345±10	340±13	344±13	338±14	330±11	332±9	330±13
TF100	350±12	348±12	344±14	347±14	342±14	347±13	351±10
TF=200	344±12	340±12	340±14	341±14	341±13	344±13	346±12
LVP (mmHg)							
NS	169±13	165±10	166±11	164±9	163±10	165±8	165±12
TF100	171±10	172±8	169±7	167±8	165±9	168±10	168±11
TF200	173±10	174±9	171±8	169±10	165±11	168±10	169±13
+dp/dtmax (mmHg/s)							
NS	3540±200	3570±240	3550±210	3520±185	3500±230	3500±210	3525±230
TF100	3600±280	3540±220	3538±242	3520±209	3522±269	3547±265	3560±275
TF200	3578±279	3578±212	3547±235	3524±237	3539±239	3542±183	3563±223
-dp/dtmax (mmHg/s)							
NS	3000±220	2950±210	3050±250	3070±190	2930±250	2900±240	2890±230
TF100	3100±215	3050±225	3000±257	2950±214	3048±253	3150±260	3010±180
TF200	3014±223	2980±245	2988±247	2989±234	3004±229	3008±219	3009±220
CO (%)							
NS	100	99.5±5.4	98.5±5.5	98.7±4.6	99.3±6.0	99.5±4.5	99.3±6.0
TF100	100	99.2±6.6	99.8±4.5	100.4±7.6	102.2±6.5	100.2±9.1	101.4±7.0
TF200	100	99.2±8.0	100.4±5.4	101.8±8.0	102.4±7.8	102.2±8.6	102.4±9.0
TPR (%)							
NS	100	100.5±5.6	100.8±5.0	101.5±6.0	101.2±6.5	99.3±8.0	100.7±5.5
TF100	100	99.4±1.3	95.2±1.3	89.4±4.4*	84.4±4.2*	87.1±3.9	94.3±3.1
TF200	100	98.1±1.3	90.8±1.5*	85.0±6.0*	82.1±5.9*	84.5±4.7*	92.7±3.7

Data are expressed as mean ± S.D., n = 10 in each group
*P < 0.05, **P < 0.01, as compared with pre-administration of drugs (0 min)

the decrease in plasma AngII, PRA was increased after TF administration. This result may indicate that in the present experiment, TF acts actually as an inhibitor of angiotensin-converting enzyme to block the conversion of AngI to AngII, thus lowering plasma concentration of AngII.

Many effective hypotensive drugs have been introduced for clinical use in recent years. Research in large populations has shown that when the blood pressure of hypertensive subjects is controlled effectively, a low rate of cardiovascular events would be observed (7). However, data from epidemiologic studies have indicated that successful control of blood pressure is achieved only in a low percentage of the hypertensive population (11). For this reason, it is important to identify new targets for the treatment of hypertension and to develop new antihypertensive drugs, which help to improve clinical antihypertensive

Table 4. Effects of TF on PRA and plasma AngII in conscious normotensive Wistar rats, RHR and SHR

	AngII (ng/ml)	PRA (ng AngI/ml/h)
Wistar rat		
NS	152 ± 34	5.5 ± 2.1
TF100	140 ± 20	6.4 ± 1.5
TF200	128 ± 27*	7.3 ± 2.9*
RHR		
NS	320 ± 69	16.5 ± 1.7
TF100	250 ± 60*	18.0 ± 1.5*
TF200	216 ± 63**#	18.5 ± 2.8*
SHR		
NS	126 ± 29	9.5 ± 1.5
TF100	102 ± 15*	10.8 ± 1.6*
TF200	90 ± 14**#	12.3 ± 2.4**#

Data are expressed as mean ± S.D., n = 10 in each group
*P < 0.05, **P < 0.01, compared with NS group
#P < 0.05, compared with TF100 group

therapy. Traditional Chinese medicine, based primarily on plant materials, has been adopted throughout much of the Western world and has become one of the fastest growing healthcare choices. TF is one of the new antihypertensive drugs extracted from natural plants and is more economical. It also has less side effects than synthetic drugs. Therefore, TF has great potential for treatment. Sixteen flavone monomers (3, 4, 6, 10) have been identified in TF, but which one of them plays the antihypertensive role in hypertensive rats should be further studied.

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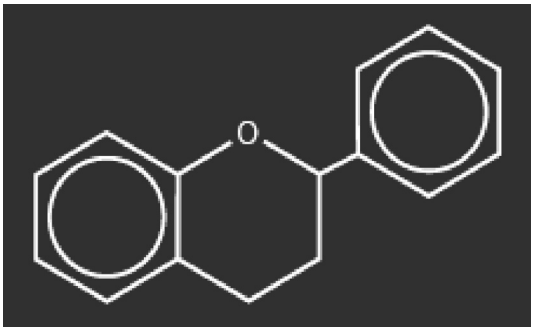

Fig.1. Structural framework of the most common flavonoids

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