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Effects of astragaloside IV on myocardial calcium transport and cardiac function in ischemic rats

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KEY WORDS astragaloside; ventricular function; calcium

ABSTRACT

AIM: To explore the effects of astragaloside IV (XGA) on myocardial calcium transport and cardiac function in ischemic rats. **METHODS:** Eighty-four Wistar rats were divided into three groups: control group (n=12); ischemic group (n=12) was given isoprenaline injection sc at a dose of 30 mg/kg; and XGA group (n=60) was given XGA after isoprenaline administration. The changes of the parameters of hemodynamics, cardiac function, and intra- and extracellular calcium concentration of the myocardial cells were determined. The dose- and time-effect relationship of XGA on myocardial calcium transport and cardiac function were observed. **RESULTS:** After XGA administration, there was significant improvement in cardiac function and hemodynamics in ischemic rats. The cardiac output, heart rate, stroke volume, mean aortic pressure, systolic aortic pressure, the stroke work of left ventricule, the right and left ventricle systolic pressure, and +dp/dt of the right ventricle of ischemic rats gradually recovered to the level of the control group with increasing the dose of XGA and prolongation of the action of XGA. The ionized calcium of the myocardium and the total amount of calcium of the myocardial tissue decreased markedly compared to those in ischemic group, and the activity of calcium pump of erythrocyte membrane increased significantly in comparison to that of ischemic group, but their changes had no trend of increase with increasing dose of the XGA. However, there was a gradual decrease of the ionized calcium of the myocardium with the prolongation of acting time of XGA. **CONCLUSION:** XGA improves the cardiac function in ischemic rats, and the reduction of excessive accumulation of intracellular calcium within myocardial cells plays an important role.

INTRODUCTION

Astragalus saponins, the main effective component of *Astragalus membranaceus*, have positive inotropic action; astragaloside IV, xylose-glucose-cyclo-astragenol (XGA), is one of the main active constituent of *astragalus* saponins[1,2]. The positive inotropic action of XGA on isolated guinea-pig papillary muscles *in vitro* had been found[2]. As the positive inotropic drugs can deteriorate cell injury of ischemic myocardium and worsen cardiac function, it is not clear whether XGA can improve the cardiac function of ischemic myocardium and there are few reports available on the action of XGA on ischemic myocardium *in vivo*. In the present studies, the effect of XGA on ischemic myocardium *in vivo* was investigated.

MATERIALS AND METHODS

Animal Eighty-four Wistar rats (42 male, cleaning grade, provided by Qingdao Animal Center, License No: Animal Quality of Shandong 200002003), body

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weight from 150 g to 200 g, were divided into three groups. Control group: 12 rats were given sterile normal saline for injection ip, and the parameters of the cardiac function and hemodynamics as well as the content of myocardial calcium in rats were measured 6 h after administration of sterile normal saline. Ischemic group: 12 rats were given isoprenaline injection sc at a dose of 30 mg/kg, and the above indexes were also measured 6 h after isoprenaline administration. XGA group: after 2 h of isoprenaline administration, 30 rats were given XGA ip at a dose of 2.5, 5.0, 10.0, 15.0, and 20.0 mg/kg, respectively; then after 6 h of isoprenaline administration, the above indexes were also measured. Another 30 rats were given XGA at a dose of 5.0 mg/kg after 2 h of isoprenaline administration, then the above indexes were determined respectively after 0, 30, 60, 90, and 120 min of XGA administration.

XGA (purity >95 %) was provided by Department of Natural Pharmacochemistry, College of Pharmacy, Shanghai Medical University (Fudan University).

Measurement of cardiac function and hemodynamics parameter[1,3,4] The right common carotid artery and the right external jugular vein of rats were separated respectively; the ductus, 1.0 mm in external diameter and 0.6 mm in internal diameter, was inserted into the left ventricle and the right ventricle through the right common carotid artery and the right external jugular vein, respectively. The left and right ventricular systolic pressure (LVSP and RVSP), the left and right ventricular end-diastolic pressure (LVEDP and RVEDP), and the maximal rise or decline rate of intraventricular pressure ($\pm dp/dt$) were recorded by RM-6000 type eight-lead physical recorder (Nihon Kohden Co, Japan) and expressed in kPa and kPa/s, respectively.

The left common carotid artery of Wistar rats was also separated, the ductus was inserted into the root of aortic artery; the systolic aortic pressure (sAP) and mean aortic pressure (mAP) was measured with RM-6000 type eight-lead physical recorder and expressed in kPa; and aortic blood flow was measured using MVF-1200 type electromagnetic flow-meter (Kaifeng Flowmeter Factory). The heart rate (HR) was obtained by II lead electrocardiography and expressed in bpm. The stroke volume (SV) and cardiac output (CO) of the left ventricule were calculated and expressed in mL/beat and mL/min. The stroke work of left ventricle (LVSW) and the systemic peripheral vascular resistance (SVR) were calculated respectively with the formula mAP×SV×7.5×13.6×10^{-3} kg·m·$beat^{-1}$ and mAP/CO×80×7.5×10^{3}dyn·s·cm^{-5}.

Measurement of myocardial calcium and the activity of membrane pump The blood was anticoagulated with heparin and used for determining the activity of calcium pump and natrium pump with erythrocyte hemolysate chemical method[5] and expressed in U, 1 U equals to 1 μmol inorganic phosphorus which released from blood hemoglobin per gram after incubating for 2 h. The content of total calcium of the myocardial tissue ($MyoCa_t$) was measured by using atomic absorption spectrophotometry and expressed in mmol/kg dry weight.

The ventricular myocardium was excised and mounted into myocardium lavage solution without calcium (NaCl 118, KCl 4.7, K_2HPO_4 0.93, $MgSO_4$ 1.2, $NaHCO_3$ 26, and glucose 5 mmol/L) , after the blood was washed out , the myocardium was cut into pieces and placed into 0.1 % calf serum albumin (BSA)1640 solution containing 2 g/L of type I collagenase, digested at 37 ℃ and gradually recalcified until a final concentration of 1.0 mmol/L was reached ,then digested for 35-40 min and an appropriate amount of 1 % BSA-1640 solution was added to terminate the digestion. Myocardial cells were collected and washed with 1 % and 4 % BSA-1640 solution (containing calcium 1.0 mmol/L), then suspended in 1 % BSA-1640 solution (the pole shape rates was nearly 75 %), then added with 1.0 mmol/L of Fura-2/AM and incubated in 37 ℃ water bath for 40 min. Washing out the remaining Fura-2/AM, the cell count was modulated to about 1.5×10^7-3.5×10^7/L, the fluorescence value (F) was determined with LS-5B fluorescence spectrophotometer (Perkin-Elmer Co, provoked wave length 340 nm, emitted wave length 550 nm), maximum fluorescence value (F_{max}) and minimum fluorescence value (F_{min}) were determined respectively after adding 0.1 % Triton-X-100 and egtazic acid 2.5 mmol/L, then the concentration of ionized calcium of the myocardium ($MyoCa_i$) was calculated with formula KD×(F-F_{min})/(F_{max}-F) nmol/L (KD is constant and equals to 224)[6].

Observation of myocardial ultrastructure A piece of ventricular myocardium was taken, fixed with 2.5 % glutaric dialdehyde and 1 % osmic acid respectively, then washed with 0.1 mmol/L phosphate buffer solution, embeded with 618 epoxy resin, solidified with dodecenyl succinic anhydride, then plasticized with dibutyl phthalate, and stained with sodium acetate, and finally their ultrastructure was observed by JEM-1200 type electron microscope.

Statistical analysis Data base was set up with SPSS10.0 software package (SPSS Inc, Chicago, IL) for analysis. Difference between groups were assessed with two-side *t* test or variance analysis, difference within groups were assessed with self-paired *t* test. A probability value less than 0.05 was considered to be significant.

RESULTS

Effects of the different dose or action time of XGA on hemodynamics and cardiac function in ischemic rats The LVSP, ±d*p*/d*t* of left and right ventricle, SV, CO, mAP, sAP, and LVSW in ischemic group decreased significantly compared to those of control group, however the RVSP, LVEDP, RVEDP, and HR increased markedly. After XGA administration, especially at a dose of 15 mg/kg and 20 mg/kg, there were significant improvement of cardiac function and hemodynamics of ischemic rats. And the CO, mAP, sAP, LVSW, HR, SV, LVSP, ±d*p*/d*t* of the left ventricle, RVSP, and +d*p*/d*t* of the right ventricle of ischemic rats gradually recovered to the level of control group with increasing dose of XGA. SVR of ischemic rats decreased gradually with the increasing dose of XGA, especially at doses of 15 mg/kg and 20 mg/kg.

Moreover, 60 min after XGA administration at a dose of 5.0 mg/kg, especially 90 min and 120 min, the significant improvement of cardiac function and hemodynamics in ischemic rats was noted. The CO, HR, SV, sAP, mAP, LVSW, LVSP, +d*p*/d*t* of the left and right ventricle, and RVSP in ischemic rats gradually recovered to the level of control group with prolonging of the action time of XGA. There was no significant change in SVR with prolongation of the action time of XGA (Tab 1, 2).

Effects of the different dose or action time of XGA on the myocardial calcium and the activity of membrane pump in ischemic rats The concentration of $MyoCa_i$ and the content of $MyoCa_t$ in ischemic group were higher than those of control groups; but the activity of calcium pump of erythrocyte membrane was lower than that of control group. After XGA administration, the concentration of $MyoCa_i$ and the content of $MyoCa_t$ in ischemics rats decreased markedly compared to those of ischemic group, but were still higher than those of control group; the activity of calcium pump of erythrocyte membrane which increased significantly compared to that of ischemic group basically recovered to the levels of control group; though the gradual decrease of the concentration of $MyoCa_i$ and the content of $MyoCa_t$ as well as the gradual increase of the activity of calcium pump of erythrocyte

Tab 1. Effects of the different dose and action time of XGA on the left and right ventricle pressure of ischemic rats. Mean±SD. $^{a}P>0.05$, $^{b}P<0.05$, $^{c}P<0.01$ *vs* control group. $^{d}P>0.05$, $^{e}P<0.05$, $^{f}P<0.01$ *vs* ischemic group. $^{g}P>0.05$, $^{h}P<0.05$, $^{i}P<0.01$ *vs* (1) group. $^{j}P>0.05$, $^{k}P<0.05$, $^{l}P<0.01$ *vs* (6) group.

Group	*n*	Left ventricle LVSP (kPa)	LVEDP (kPa)	+d*p*/d*t* (kPa·s^{-1})	-d*p*/d*t* (kPa·s^{-1})	Right ventricle RVSP (kPa)	RVEDP (kPa)	+d*p*/d*t* (kPa·s^{-1})	-d*p*/d*t* (kPa·s^{-1})
Control	12	12.69±0.24	0.9±0.4	437±16	233±14	3.2±0.6	0.3±0.3	128±11	67±16
Ischemic	12	10.8±0.8^{c}	1.5±0.4^{c}	369±24^{c}	211±18^{c}	5.9±0.8^{c}	0.9±0.5^{c}	81±10^{c}	42±15^{c}
XGA									
Dose/mg·kg^{-1}									
(1) 2.5	6	12.8±0.3^{af}	1.4±0.3^{bd}	388±15^{cd}	221±12^{ad}	5.1±0.4^{ce}	0.6±0.3^{bd}	94±14^{ce}	58±12^{ae}
(2) 5.0	6	12.82±0.25^{afg}	1.2±0.4^{adg}	401±17^{ceg}	231±14^{aeg}	4.5±0.5^{cfg}	0.44±0.21^{aeg}	109±17^{bfg}	60±14^{aeg}
(3) 10.0	6	13.90±0.28^{cfi}	0.9±0.4^{aeg}	436±14^{afi}	242±11^{afi}	3.3±0.5^{afi}	0.34±0.26^{aeg}	125±13^{afi}	69±11^{afg}
(4) 15.0	6	13.75±0.24^{cfi}	0.9±0.4^{aeg}	452±19^{afi}	244±12^{afi}	3.1±0.5^{afi}	0.31±0.28^{aeg}	131±15^{afi}	73±12^{afg}
(5) 20.0	6	14.0±0.27^{cfi}	0.8±0.4^{afh}	449±18^{afi}	239±13^{afh}	3.3±0.5^{afi}	0.32±0.24^{aeg}	129±13^{afi}	68±12^{afg}
Time/min									
(6) 0	6	10.3±0.6^{cd}	1.5±0.4^{bd}	358±19^{cd}	208±16^{cd}	5.8±0.6^{cd}	0.8±0.4^{cd}	85±13^{cd}	44±11^{cd}
(7) 30	6	10.7±0.6^{cdj}	1.4±0.4^{bdj}	364±20^{cdj}	210±15^{cdj}	5.5±0.6^{cdj}	0.8±0.3^{cdj}	88±12^{cdj}	46±13^{bdj}
(8) 60	6	11.7±0.4^{cel}	1.2±0.4^{adj}	389±14^{cdl}	225±15^{adj}	4.9±0.7^{cek}	0.5±0.3^{adj}	101±10^{cfk}	57±12^{aej}
(9) 90	6	12.7±0.5^{afl}	1.0±0.4^{aek}	409±17^{cfl}	244±17^{afl}	4.5±0.6^{cfl}	0.37±0.28^{aek}	118±12^{afl}	64±14^{aek}
(10) 120	6	12.5±0.3^{afl}	1.0±0.4^{aek}	412±15^{cfl}	242±13^{afl}	4.4±0.4^{cfl}	0.36±0.25^{aek}	116±13^{afl}	63±12^{aek}

Tab 2. Effects of the different dose and action time of XGA on cardiac function in ischemic rats. Mean±SD. [a]*P*>0.05, [b]*P*<0.05, [c]*P*<0.01 *vs* control group. [d]*P*>0.05, [e]*P*<0.05, [f]*P*<0.01 *vs* ischemic group. [g]*P*>0.05, [h]*P*<0.05, [i]*P*<0.01 *vs* (1) group. [j]*P*>0.05, [k]*P*<0.05, [l]*P*<0.01 *vs* (6) group.

Group	*n*	HR (bpm)	sAP (kPa)	mAP (kPa)	SV ($mL \cdot beat^{-1}$)	CO ($mL \cdot min^{-1}$)	LVSW ($kg \cdot m \cdot beat^{-1}$)	SVR ($dyn \cdot s \cdot cm^{-5}$)
Control	12	290±10	13.0±1.2	10.5±1.0	0.85±0.09	250±20	0.91±0.08	25 200±629
Ischemic	12	380±12[c]	11.0±1.1[c]	8.7±1.2[c]	0.57±0.10[c]	208±25[c]	0.51±0.09[c]	25 096±720[a]
XGA								
Dose/$mg \cdot kg^{-1}$								
(1) 2.5	6	352±5[cf]	12.1±0.7[ae]	9.4±0.8[bd]	0.65±0.08[cd]	229±14[bd]	0.62±0.07[ce]	24 629±672[ad]
(2) 5.0	6	336±7[cfi]	12.6±0.6[afg]	9.9±1.1[adg]	0.74±0.07[bfg]	246±16[afg]	0.75±0.09[ceh]	24 146±534[ceg]
(3) 10.0	6	316±7[cfi]	13.8±0.5[afi]	10.8±0.7[afi]	0.86±0.05[afi]	269±15[afi]	0.95±0.06[afi]	24 089±698[ceg]
(4) 15.0	6	292±8[afi]	13.7±0.4[afi]	11.0±0.7[afi]	0.95±0.07[bfi]	279±19[bfi]	1.06±0.10[cfi]	23 656±670[cfh]
(5) 20.0	6	265±10[cfi]	13.9±0.5[afi]	10.9±0.9[afi]	1.10±0.08[cf i]	294±20[cfi]	1.22±0.13[cfi]	22 244±710[cfi]
Time/min								
(6) 0	6	401±14[cf]	10.4±0.9[cd]	8.4±1.1[cd]	0.54±0.08[cd]	200±18[cd]	0.46±0.07[cd]	25 200±611[ad]
(7) 30	6	384±13[cdj]	10.9±1.0[cdj]	8.9±0.9[cdj]	0.58±0.08[cdj]	208±15[cdj]	0.53±0.08[cdj]	25 673±654[adj]
(8) 60	6	366±10[cel]	11.6±0.7[bdk]	9.2±0.7[bdj]	0.68±0.09[cek]	219±14[cdj]	0.64±0.06[cfl]	25 205±598[adj]
(9) 90	6	341±9[cfl]	12.8±0.7[afl]	9.9±0.8[aek]	0.79±0.10[afl]	237±16[ael]	0.80±0.09 [cfl]	25 063±688[adj]
(10) 120	6	327±12[cfl]	12.6±0.8[afl]	10.1±1.1[aek]	0.78±0.09[afl]	244±15[afl]	0.80±0.07[cfl]	24 836±613[adj]

membrane with increasing of the dose of XGA occurred, no significance was found (P>0.05), except for the concentration of $MyoCa_i$ after XGA administration at a dose of 20.2 mg/kg (Tab 3).

Furthermore, after 90 min of XGA administration at a dose of 5.0 mg/kg, the concentration of $MyoCa_i$ and the content of $MyoCa_t$ in ischemic rats began to decrease in comparison to those of ischemic group, but were still higher than those of control group; and the gradual decrease of the $MyoCa_i$ concentration with prolonging of action time of XGA could be found (P<0.05 or 0.01). Though the gradual decrease of the content of $MyoCa_t$ was also found, not significant (P>0.05). The activity of calcium pump of erythrocyte membrane increased significantly compared to that of ischemic group after 90 min and 120 min of XGA administration, and basically recovered to the control group levels, however no time-effect was found. No significant change occurred to the activity of natrium pump before and after XGA administration.

Myocardium ultrastructure After XGA administration, the edema of myocardial interstitium, mitochondria, and sacroplasmic reticulum alleviated in some degree; necrosis of myocardium disappeared; the sacromere of myocardium became normal basically, and the density of mitochondria increased (Fig 1). After XGA administration at a dose of 5.0 mg/kg, no improvement of the myocardial ultrastructure occurred within 90 min of XGA administration, however the re-

Tab 3. Results of the myocardial calcium and the activity of membrane pump in three groups. Mean±SD. [a]*P*>0.05, [b]*P*<0.05, [c]*P*<0.01 *vs* control group. [d]*P*>0.05, [e]*P*<0.05, [f]*P*<0.01 *vs* ischemic group. [g]*P*>0.05, [h]*P*<0.05, [i]*P*<0.01 *vs* (1) group. [j]*P*>0.05, [k]*P*<0.05, [l]*P*<0.01 *vs* (6) group.

Group	*n*	$MyoCa_i$ ($nmol \cdot L^{-1}$)	$MyoCa_t$ ($mmol \cdot kg^{-1}$ dry weight)	Ca-pump (U)	Na-pump (U)
Control	12	121±12	4±1	165±29	67±30
Ischemic	12	468±133[c]	12±4[c]	124±50[b]	53±22[a]
XGA					
Dose/$mg \cdot kg^{-1}$					
(1) 2.5	6	240±42[cf]	7±2[ce]	175±40[ae]	64±28[ad]
(2) 5.0	6	227±50[cfg]	6±2[cfg]	182±38[aeg]	66±29[adg]
(3) 10.0	6	214±48[cfg]	6±2[cfg]	194±35[afg]	62±30[adg]
(4) 15.0	6	200±46[cfg]	5±2[cfg]	190±41[aeg]	60±25[adg]
(5) 20.0	6	185±40[cfh]	6±1[cfg]	206±50[bfg]	66±26[adg]
Time/min					
(6) 0	6	479±64[cd]	12±7[cd]	118±49[bd]	49±24[ad]
(7) 30	6	438±53[cdj]	10±5[cdj]	127±43[bdj]	54±26[adj]
(8) 60	6	381±69[cdk]	9±5[cdj]	154±54[adj]	64±23[adj]
(9) 90	6	241±57[cfl]	6±2[cfj]	179±37[aej]	66±27[adj]
(10) 120	6	231±49[cfl]	6±3[bfj]	187±55[aek]	65±30[adj]

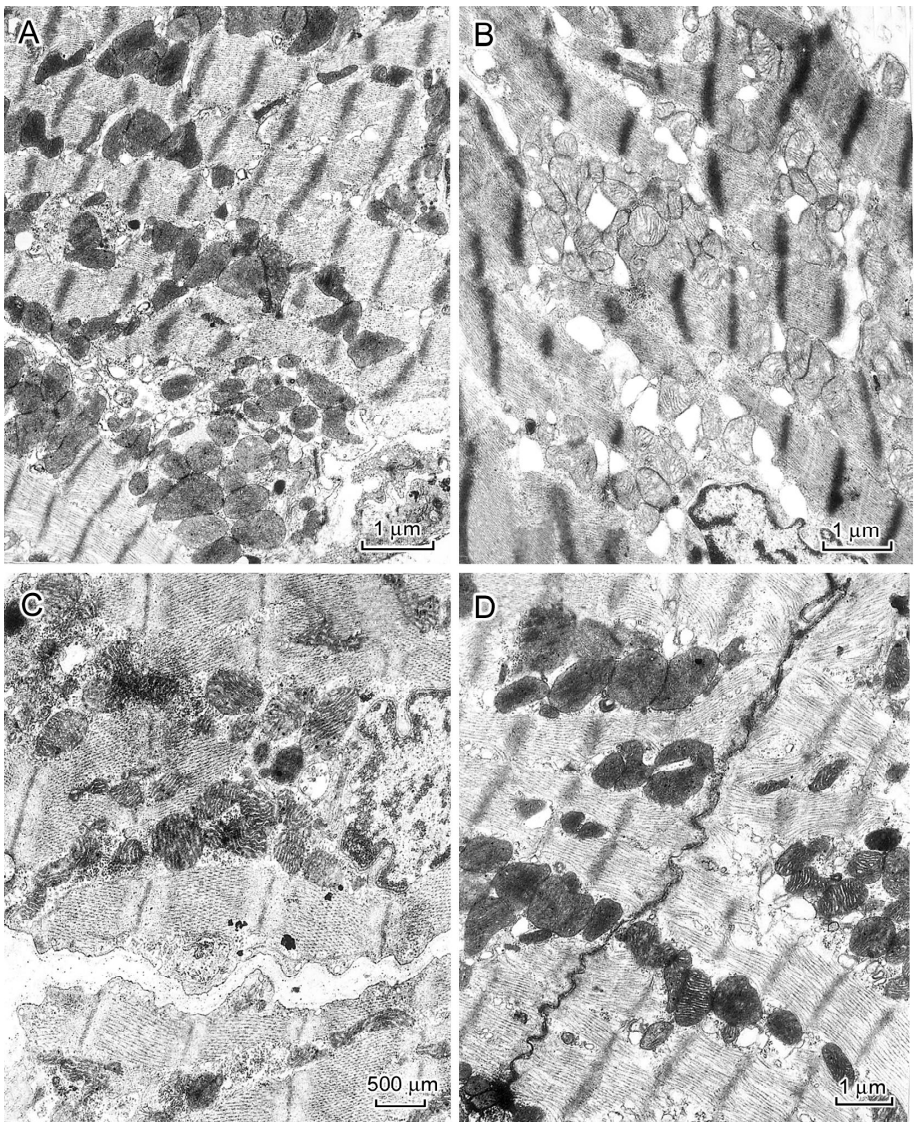

Fig 1. Myocardium ultrastructure of rats: A (×7500): the normal rats; B (×6000): the ischemic rats; C (×10000): ischemic rats after XGA administration at a dose of 10 mg/kg; D (×5000): ischemic rats after XGA administration at a dose of 20 mg/kg.

markable improvement of the myocardial ultrastructure developed after 90 min and 120 min of XGA administration.

DISCUSSION

Astragalus membranaceus, one of the most common Chinese traditional medicine, had multiple functions, including the effect of buqi, reducing blood pressure, increasing the cardiac contractility, diminishing inflammation, and alleviating edema, *etc*. According to the rule of Chinese pharmacopeia, the dry root of *Astragalus membranceus* (Fisch) Bge *or Astragalus mongholicus* (Bye) Hsiao were served as drugs. The major components of *Astragalus membranaceus* included mainly *astragalus* saponins, polysaccharose, amino acid, flavone, and muti-trace elements[7,8]. As the main component of *Astragalus membranaceus* exerting effect on cardiovascular system, *astragalus* saponins owned about ten components and the majorities were XGA. The contents of XGA were 0.095 mg to 1.40 mg per gram crude *Astragalus membranaceus,* about 0.086 % -2.4 % of total *astragalus* saponins[7-9]. The study on pharmacological effects of XGA will provide a theoretical basis for the drug exploiting of the monomer of *Astragalus membranaceus* .

Zhong[1] *et al* and Zhang[10] *et al* found total *astragalus* saponins had positive inotropic action and antioxidation, although Chu *et al*[11] proved to be the otherwise. The explaination for the controversy lies in

the various ingredients of *astragalus* saponins and their different regions of *Astragalus membranaceus* where it was planted. At present there was very few studies on the action of the monomer of *astragalus* saponins available. Shen *et al*[2] found positive inotropic action of XGA *in vitro*; Luo *et al*[12] further illustrated its effect on improvement of left ventricular modelling and ejection function in patients with congestive heart failure after continuous administration of XGA injection for two weeks. In our studies, it was found that XGA could significantly improve the parameters of cardiac function and hemodynamics in ischemic rats *in vivo*, and some of the improvement was dose-dependent. In addition, the effect of XGA on ischemic myocardium varied with the action time of XGA at a dose of 5.0 mg/kg and was remarkable after 90-120 min of XGA administration. These facts were consistent with the changes of myocardial ultrastructure in ischemic rats after XGA administration, implying that XGA did not aggravate the cell injury of ischemic myocardium and on contrary, the cardiac function was improved after its administration.

The mechanism of XGA underlying the improvement of cardiac function was unclear. That the lack of involvement of intracellular calcium in the action of XGA had been indicated[2], and it was postulated that the positive inotropic action exerted by XGA involved in affecting the potentiation of calmodulin in combination with calcium; but there was no reports regarding the direct determination of intracellular calcium of myocardium. In our studies, the concentration of $MyoCa_i$ and the content of $MyoCa_t$ in ischemic rats was measured directly and was found to be decreased markedly after XGA administration. The reduction of the $MyoCa_i$ concentration was related to the action time of XGA, indicating that improvement of the cardiac function in ischemic rats by XGA might be caused by alleviation of excessive accumulation of calcium within the myocardial cells. Moreover, the activity of calcium pump in erythrocyte membrane increased significantly compared to that of ischemic group after XGA administration in our studies. As the activity of erythrocyte membrane pump was consistent with that of myocardial cell membrane[13], the changes in the activity of calcium pump in erythrocyte membrane could reflect the changes in myocardial cell membrane, thus the increase of the calcium pump activity could contribute to the increase of the removal of the intracellular calcium and the reduction of excessive accumulation of calcium within the ischemic myocardial cells, and finally the ischemic myocardial function was improved. We also found no significant changes in the natrium pump activity in erythrocyte membrane before and after administration XGA, which suggested that the reduction of intracellular calcium concentration of myocardium was not associated with the action of XGA on the activity of natrium pump of myocardial cell membrane.

A limitation of our study results from the inability to obtain adequate samples owing to the small sample size and from inability to determine the activity of calcium pump of sacroplasmic reticulum membrane, meanwhile the purity of XGA is not reach to 100 % and there are some factors to affect the parameters of cardiac function and hemodynamics. Thus further systematic studies are needed to define the action of XGA on ischemic myocardium.

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