Effects of Astragaloside Ⅳ Derivative on Heart Failure in Rats

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Abstract: **Objective** Astragaloside Ⅳ derivative (ASId) is one of Astragaloside Ⅳ (ASI) derivatives with higher water-solubility and may have more druggability than ASI. The present study aims at observing the effects of ASId on cardiovascular parameters in chronic heart failure in rats. **Methods** Using echocardiographic and haemodynamic measurements, the effects of ASId on congestive heart failure (CHF) induced by ligation of the left coronary artery in rats were investigated. **Results** ASId iv 0.5, 1.0, and 2.0 mg/(kg·d) attenuated the decline of ejection fraction. The peak derivatives of the left ventricle (LV) pressure (d*p*/d*t*) in ASId treated groups were significantly increased. Both LV volumes in diastole and in systole were decreased significantly after ASId treatment, accompanied with a trend towards normalization of relative wall thickness at end-systole. ASId 0.5, 1.0, and 2.0 mg/(kg·d) attenuated the increase of LV systolic and diastolic wall stress. ASId treatment also inhibited compensatory hypertrophy of depressed heart. **Conclusion** ASId could improve cardiac functions and inhibite compensatory hypertrophy and LV remodelling, which suggests the possibility of ASId as a new therapeutic drug for the treatment of CHF.

Key words: astragaloside IV derivative, heart failure, rat

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Introduction

Heart failure (HF) is one of the main causes of mortality around the world, myocardial infarction (MI) frequently produces left ventricular (LV) dilation with hypertrophy (LV remodelling), which leads to depressed cardiac performance (Pfeffer and Braunwald, 1990).

Radix Astragali is a Chinese herbal medicine that has been reported to improve the LV function and mitigate the cardiac hypertrophy in HF patients (Yuan, 2003; Li *et al*, 2003), and evidence showed that astragaloside IV (ASI) is the active ingredient of *Radix Astragali* for the treatment of HF (Wu, 2005; Zheng *et al*, 2005; Liu *et al*, 2001; Liu and Shen, 2001). ASI could increase systolic and diastolic functions in acute HF of canine induced by pentobarbital sodium and improve the cardiac depressant effect induced by Propranolol. Our previous studies showed that ASI could improve cardiac functions, inhibit compensatory hypertrophy of myocardial cells, and lower the number of apoptotic myocytes (Zhao *et al*, 2009). But ASI has lower water-solubility, which restricts in dosage for pharmacodynamics and disadvantage for future clinical use as well.

Astragaloside Ⅳ derivative (ASId) is one of ASI derivatives by hydrolysis with higher water-solubility (Fig. 1), which can solve the problem of pharmaceutical preparation; therefore, the dosage of ASId for animal administration can be further increased. The maximum dosage of ASI for rat administration was 1 mg/kg restricted to delivery volume (usually given 1 mL per animal), while for ASId, the dosage can be given minimum 2 mg/kg to rat, ASId may improve the efficacy with lower toxicity. As we all know, water has lower toxicity as a solvent than any other cosolvent, so ASId may have more druggability than ASI. Our unpublished study showed that ASId could also improve systolic and diastolic functions, which was similar to ASI in isolated heart of rat. However, the effects of ASId on HF have not been elucidated. The aim of this study was to observe the effects of ASId on HF in rats.

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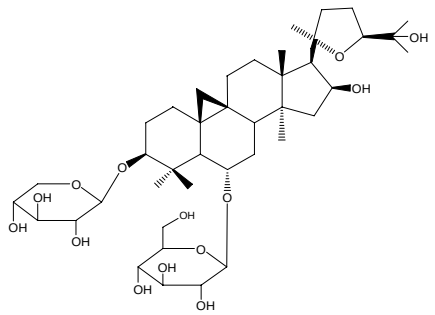
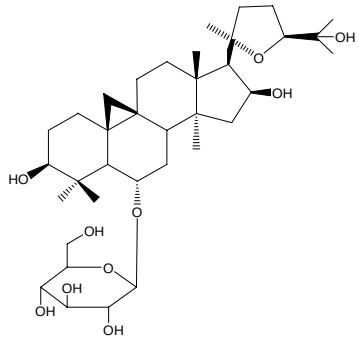


ASI ($C_{41}H_{68}O_{14}$ = 784)



ASId ($C_{36}H_{60}O_{10}$ = 652)

Fig. 1 Chemical structures of ASI and ASId

Materials and methods

Animals and reagents

Wistar male rats weighting (240–311) g were housed at (22 ± 2) ℃ in a temperature-regulated room equipped with an automatic 12/12 h photoperiod and were provided with food and water. ASId (Patent No. 200810004380.0, Batch No. 20070929) was provided by Tianjin Institute of Pharmaceutical Research. ASId was dissolved in normal saline (NS). Quinapril as the control medicine was supplied by the Shanghai Institute of Pharmaceutical Industry (Certificate No. 051).

Induction of MI and medications

MI in Wistar rats was induced by coronary artery ligation. Through a thoracotomy on the left side, the heart was exteriorized and extensive MI was induced by ligation of the left coronary artery according to previous literature (Tonnessen *et al*, 1997). Animals in the Sham group were subjected to the same surgical procedure, with the exception that the coronary artery was not ligated. Echocardiography was performed three weeks later in the surviving rats anaesthetized by ether. Those with HF according to echocardiographic criteria were randomly divided into five groups (Burrell, Chan, and Phillips, 1996; Francis, 2001), eight animals in each group. Group one (model) received NS; groups two to four received ASId 0.5, 1.0, and 2.0 mg/kg, respectively; group five received Quinapril 1.0 mg/kg. The animals in the Sham group were given NS (1 mL/kg). Drug administration was started three weeks after coronary ligation by iv injection once a day for 14 d.

Echocardiography

We used a commercially available echocardiography (VIVID 3 Pro, GE, USA) equipped with a 10 MHz linear transducer. The animal's chest was shaved and the animal was placed in supine position. The transducer was placed gently on the left. Two-dimensional (2D) images of the LV were obtained in both parasternal long-axis and short-axis views at a frame rate of 130 Hz. M-mode tracings were recorded at the level of the papillary muscles with 2D guidance. LV wall thickness and cavity diameters were measured outside the infarcted area by M-mode through the largest diameter of the ventricle. Each image consisted of 24 consecutive heart cycles and the focus area was 0–40 mm from the transducer. Ejection fraction (EF) was calculated according to the following formula:

$$EF = [(EDV - ESV) / EDV] \times 100\%$$

Where EDV is the end-diastolic volume in LV and ESV is the end-systolic volume in LV

LV internal diameters were measured according to the guidelines of the American Society of Echocardiography (ASE) (Luo *et al*, 1995).

$$\text{Relative wall thickness at end-systole (RWTs)} = [(IVSs + LVPWs) / LVIDs] \times 100\%$$

Where IVSs is interventricular septum thickness at end-systole and LVPWs is posterior wall thickness at end-systole, LVIDs is LV internal diameter at end-systole

$$\text{LV systolic wall stress} = 1.36 \times (\text{aortic systolic pressure} \times LVIDs) / (IVSs + LVPWs)$$

$$\text{LV diastolic wall stress} = 1.36 \times (LVEDP \times LVIDd) / (IVSd + LVPWd)$$

Where LVEDP is left ventricular end-diastolic pressure
IVSd is interventricular septum thickness at end-diastole
LVPWd is LV posterior wall thickness at end-diastole
LVIDd is LV internal diameter at end-diastole

Haemodynamic measurements

An F-2 (0.7 mm in internal diameters) catheter with pressure-transducer was inserted through the right carotid artery into the aorta to determine systolic blood pressure (SBP). The catheter was subsequently

advanced further into the LV, and end-diastolic pressure (LVEDP) and LV systolic pressure (LVSP) were recorded. The transducer was connected to a data acquisition workstation (model MP150, BIOPAC Systems, USA) to a computer running Acknowledge (version 3.7.1). Peak positive and peak negative first derivatives of the LV pressure (+LVd*p*/d*t* and –LVd*p*/d*t*) were calculated with the Acknowledge software.

Cardiac mass index measurements

After haemodynamic measurements, the rats were subsequently euthanized by excision of the heart. The heart was weighted, and the infarcted area (scar tissue) was excised from the non-infarcted LV. Then the infarction size was estimated by weighing the excised scar tissue. The right tibia of each rat was isolated and then its length was measured (Zhang *et al*, 2007).

Statistical analysis

Data are expressed as mean ± SD. The statistical significance among groups was evaluated by a one-way analysis of variance (ANOVA) with SAS software (version 9.0, SAS INSTITUTE INC, USA). $P < 0.05$ is considered to be statistically significant.

Results

Effects of ASId on the LV functions

The EF in the model group was markedly decreased by 30% compared with normal baseline three weeks after coronary artery ligation, and further decreased after administration of NS for another two weeks. The values of +d*p*/d*t* and –d*p*/d*t* were decreased by 29% and 26%, respectively. After administration of ASId 0.5, 1.0, and 2.0 mg/(kg·d), the decline of the EF was attenuated ($P < 0.01$–0.001). The +d*p*/d*t* in all ASId-treated groups were significantly increased ($P < 0.01$–0.001), and the –d*p*/d*t* value was significantly increased ($P < 0.01$) compared with the model group after administration of ASId at a dose of 2.0 mg/(kg·d) (Table 1 and Fig. 2).

Effects of ASId on LV geometry in rats with HF

LV geometry in various groups was analyzed with echocardiography. The results demonstrated the progression of LV remodelling (dilatation of LV volume and attenuation of RWTs) during a 5-week period after coronary artery ligation in the absence of treatment. ASId also normalised the RWTs at end-systole in a dose-dependent manner and LV volume decreased significantly two weeks after ASId treatment (Tables 2 and 3).

Table 1 Effects of ASId on EF

Groups	Dose / (mg·kg^{-1}·d^{-1})	Normal / %	before treatment / %	After treatment / %	
				1 week	2 weeks
Sham	—	94.3±1.4	94.3±1.0	94.9±1.7	94.9±2.2
				(0.6±2.0)	(0.6±2.0)
Model	—	93.6±3.0	64.9±6.9$^{\triangle\triangle\triangle}$	61.6±8.7$^{\triangle\triangle\triangle}$	58.5±15.6$^{\triangle\triangle\triangle}$
				(-3.3±2.5)	(-6.4±12.6)
ASId	0.5	93.9±2.9	69.6±5.2	77.1±6.0	78.0±7.8
				(7.5±5.2***)	(8.4±8.5**)
	1.0	92.3±3.1	62.6±6.3	73.9±7.4	73.4±7.4
				(11.3±2.6$^{***\$}$)	(10.8±7.0**)
	2.0	93.3±2.4	63.9±10.5	76.8±9.1	75.9±5.4
				(12.9±7.5***)	(12.0±8.3***)
Quinapril	1.0	94.3±1.7	66.8±11.0	72.5±6.5	72.5±6.3
				(5.8±6.5***)	(5.8±9.2*)

Note: Values are mean ± SD in each group ($n = 8$)

$^{\triangle\triangle\triangle}P < 0.001$: statistical significance over the Sham group (comparison of group means after ANOVA)

$^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$: statistical significances over the model group (comparison of group means after ANOVA)

$^{\$}P < 0.05$: statistical significance between ASId 1.0 mg/(kg·d) and Quinapril treatment

EF gain values compared to before treatment are available in brackets

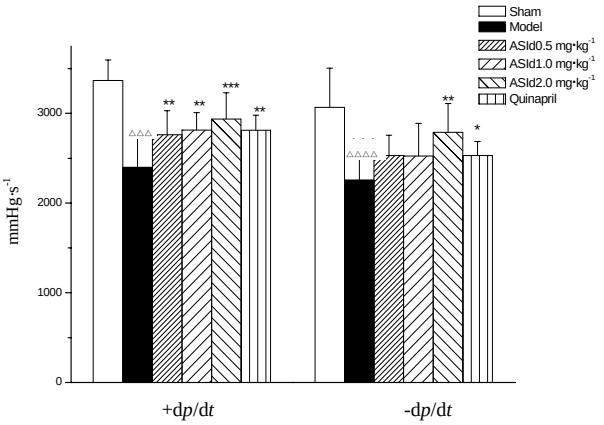


Fig. 2 Effects of ASId on ＋d*p*/d*t* and –d*p*/d*t*

Note: Values are mean ± SD in each group ($n = 8$)

$^{\triangle\triangle\triangle}P < 0.001$: statistical significance over the Sham group (comparison of group means after ANOVA)

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$: statistical significances over the model group (comparison of group means after ANOVA)

No significance between ASId 1.0 mg/(kg·d) and Quinapril treatment

Effects of ASId on haemodynamics in rats with HF

LVSP in the model group was 13% lower than that in the Sham group, while LVEDP in the model group was increased markedly, suggesting severe cardiac dysfunction in the CHF rats after coronary artery ligation.

Table 2 Effects of ASId on RWTs

Groups	Dose / ($mg \cdot kg^{-1} \cdot d^{-1}$)	Normal / %	Before treatment / %	After treatment / %	
				1 week	2 weeks
Sham	—	199.2±21.5	219.1±27.2	230.9±45.1	247.3±54.1
				(11.8±51.2)	(28.2±43.8)
Model	—	206.9±35.0	72.89±17.5$^{\triangle\triangle\triangle}$	65.7±17.3$^{\triangle\triangle\triangle}$	61.0±18.6$^{\triangle\triangle\triangle}$
				(-7.2±9.3)	(-11.9±15.9)
ASId	0.5	211.1±37.1	86.6±13.1	98.3±23.1	102.7±30.7
				(11.8±13.4**)	(16.2±24.9*)
ASId	1.0	190.8±69.1	72.0±12.2	95.2±18.8	99.4±22.5
				(23.2±9.8***)	(27.4±21.8***)
ASId	2.0	202.9±39.8	72.2±16.1	104.5±36.3	102.9±20.7
				(32.3±25.2***)	(30.7±12.7***)
Quinapril	1.0	215.8±37.7	75.9±20.5	88.6±19.2	85.5±11.3
				(12.7±17.3**)	(9.6±18.9*)

Note: Values are mean ± SD in each group (n = 8)

$^{\triangle\triangle\triangle}P < 0.001$: statistical significance over the Sham group (comparison of group means after ANOVA)

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$: statistical significances over the model group (comparison of group means after ANOVA)

NO statistical significance between ASId 1.0 mg/(kg·d) and Quinapril

RWTs gain values compared to before treatment are available in brackets

Table 3 Effects of ASId on LV volume

	Groups	Dose / ($mg \cdot kg^{-1} \cdot d^{-1}$)	Normal / %	Before treatment / %	After treatment / %	
					1 week	2 week
	Sham	—	0.76 ± 0.19	0.76 ± 0.18	0.77 ± 0.15	0.66 ± 0.16
					(0.01 ± 0.07)	(-0.10 ± 0.18)
	Model	—	0.58 ± 0.19	1.50 ± 0.38$^{\triangle\triangle\triangle}$	1.55 ± 0.51$^{\triangle\triangle\triangle}$	1.61 ± 0.54$^{\triangle\triangle\triangle}$
					(0.04 ± 0.33)	(0.11 ± 0.29)
EDV (cm^3)	ASId	0.5	0.59 ± 0.11	1.47 ± 0.43	1.44 ± 0.52	1.40 ± 0.49
					(-0.03 ± 0.32)	(-0.07 ± 0.39)
	ASId	1.0	0.76 ± 0.19	1.38 ± 0.19	1.33 ± 0.30	1.17 ± 0.39
					(-0.05 ± 0.38)	(-0.21 ± 0.36*)
	ASId	2.0	0.67 ± 0.16	1.49 ± 0.37	1.23 ± 0.25	1.14 ± 0.34
					(-0.26 ± 0.30)	(-0.35 ± 0.25**)
	Quinapril	1.0	0.64 ± 0.12	1.50 ± 0.33	1.39 ± 0.32	1.30 ± 0.22
					(-0.11 ± 0.22)	(-0.20 ± 0.21*)
	Sham	—	0.05 ± 0.02	0.05 ± 0.02	0.04 ± 0.02	0.04 ± 0.02
					(-0.01 ± 0.02)	(-0.01 ± 0.02)
	Model	—	0.04 ± 0.02	0.55 ± 0.21$^{\triangle\triangle\triangle}$	0.61 ± 0.29$^{\triangle\triangle\triangle}$	0.68 ± 0.40$^{\triangle\triangle\triangle}$
					(0.07 ± 0.15)	(0.13 ± 0.29)
ESV (cm^3)	ASId	0.5	0.04 ± 0.02	0.43 ± 0.22	0.33 ± 0.16	0.32 ± 0.19
					(-0.10 ± 0.09*)	(-0.11 ± 0.20*)
	ASId	1.0	0.06 ± 0.03	0.50 ± 0.16	0.37 ± 0.15	0.33 ± 0.18
					(-0.13 ± 0.11**)	(-0.16 ± 0.12**)
	ASId	2.0	0.05 ± 0.02	0.56 ± 0.28	0.3 ± 0.14	0.29 ± 0.14
					(-0.26 ± 0.21***)	(-0.28 ± 0.20***)
	Quinapril	1.0	0.04 ± 0.01	0.53 ± 0.24	0.40 ± 0.17	0.38 ± 0.13
					(-0.13 ± 0.14**)	(-0.15 ± 0.16**)

Note: Values are mean ± SD in each group (n = 8)

$^{\triangle\triangle\triangle}P < 0.001$: statistical significance over the Sham group (comparison of group means after ANOVA)

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$: statistical significance over the model group (comparison of group means after ANOVA)

No statistical significance between ASId 1.0 mg/(kg·d) and Quinapril treatments (comparison of group means after ANOVA)

LV volume gain values compared to before treatment are available in brackets

ASId 0.5, 1.0, and 2.0 mg/(kg·d) attenuated the increase of LVEDP. The rats in the model group displayed higher systolic and diastolic wall stress of LV. Compared with the model group, ASId (0.5, 1.0, and 2.0 mg/kg/day) significantly lowered the systolic wall stress by 34% ($P < 0.01$), 36% ($P < 0.01$), 45% ($P < 0.001$), and lowered the diastolic wall stress by 84% ($P < 0.001$), 89% ($P < 0.001$), 93% ($P < 0.001$), respectively (Fig. 3).

Effects of ASId on cardiac mass index in rats with HF

Heart weight-to-tibial length ratios were decreased significantly after ASId 1.0 and 2.0 mg/(kg·d) treatment compared with the model group (Fig. 4).

Discussion

In the present study, the effects of ASId on the post-MI HF of rats were examined. To our knowledge, this study shows for the first time that ASId could improve cardiac function in a dose-dependent manner three weeks after induction of MI, as shown by a maximum 30% increase of EF with improved LV d*p*/d*t*, which was accompanied with reduction in LVEDP, LV size, and a restoration of RWTs. At the same time, ASId ameliorated LV compliance with reduction in wall stress, and ASId has beneficial effects on hypertrophy in the CHF rats.

ASI is the effective component in *Radix Astragali* to treat HF. Our previous studies showed that ASI could improve cardiac functions with increase of fractional shortening and d*p*/d*t*, inhibite of compensatory hypertrophy, and reduce of the number of apoptotic myocytes (Zhao *et al*, 2009). ASI has lower water-solubility, which was a restriction in dosage for pharmacodynamics, together with disadvantage for future clinical use. ASId is an ASI derivative by hydrolysis with higher water-solubility and maybe with a better druggablity.

Both ASI and ASId could improve contractile functions (FS, EF, and +d*p*/d*t* indicating the systolic functions) at the same dosage (1 mg/kg), FS and +d*p*/d*t* increased by 12.9% and 38.4%, respectively after ASI administration for two weeks, while EF, +d*p*/d*t* increased by 17.3% and 17.4%, respectively after ASId administration for two weeks. The diastolic functions were also improved (–d*p*/dt and LVEDP indicating the diastolic

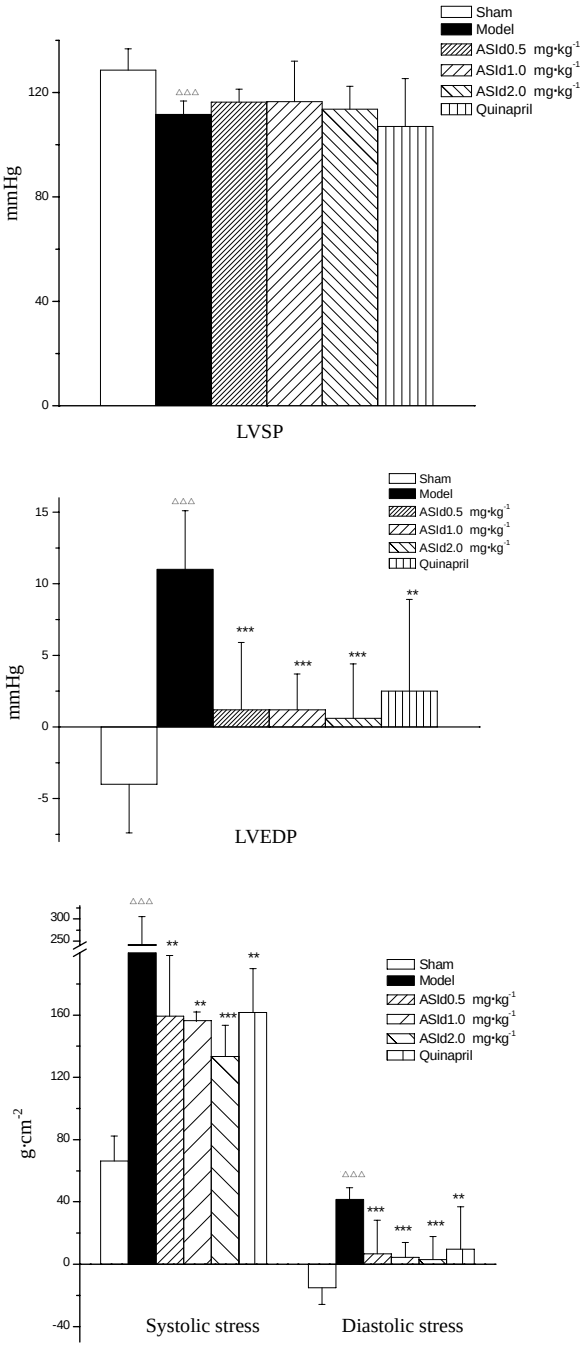


Fig. 3 Effects of ASId on LVSP, LVEDP, and wall stress

Note: Values are mean ± SD in each group ($n = 8$)

$^{\triangle\triangle\triangle}P < 0.001$: statistical significance over the Sham group (comparison of group means after ANOVA)

$^{**}P < 0.01$, $^{***}P < 0.001$: statistical significances over the model group (comparison of group means after ANOVA)

No statistical significance between ASId 1.0mg/(kg·d) and Quinapril treatments (comparison of group means after ANOVA)

functions), –d*p*/d*t* and LVEDP were improved by 44.4%, 55.4% and 12%, 89.1%, respectively after ASI and ASId administration. LV geometry ameliorated after both drugs administration, LV internal diameters at end-diastole and end-systole (LVIDd, LVIDs) decreased by

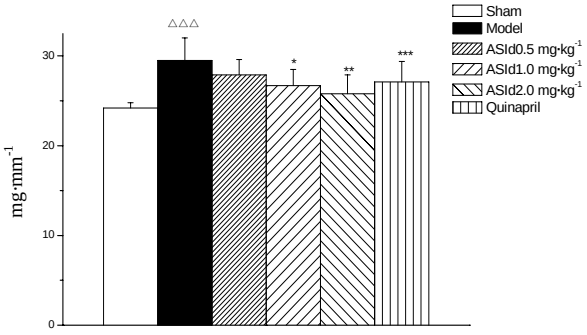


Fig. 4 Effects of ASd on cardiac mass index

Note: Values are mean ± SD in each group (n = 8)

$^{\triangle\triangle\triangle}P < 0.001$: statistical significance over the Sham group (comparison of group means after ANOVA)

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$: statistical significances over the model group (comparison of group means after ANOVA)

No statistical significance between ASId 1.0 mg/(kg·d) and Quinapril treatments (comparison of group means after ANOVA)

6.3% and 13.6%, while the LV volume at end-diastole and end-systole (EDV, ESV) decreased by 15.2% and 34.0%. At the same time, LV systolic wall stress and cardiac hypertrophy were decreased by 22.2%, 18.8% and 35.5%, 9.5%, respectively after ASI and ASId administration (Zhao *et al*, 2009). Those results suggested that ASId exhibited the similar activity for treatment of HF with ASI.

MI-induced HF has the characteristic of LV dilation with hypertrophy. The changes of LV wall thickness measured by echocardiography were influenced by both hypertrophy (concentric or eccentric hypertrophy) and cardiac function (contractility or relaxation). So, the thickness of intervillous septum and left ventricular posterior wall at systole and diastole after HF has different manifestations. In model group of this study, the IVSd was thickened, the IVSs and LVPWs were attenuated, and there was no influence on LVPWd (data not shown), which were in consistent with our previous findings (Wang *et al*, 2006). The attenuation of hypertrophy and LV remodelling maybe one of the mechanisms for ASId treatment of CHF. In addition, other mechanisms, such as antiapoptosis, targets for antihypertrophy, and inflammatory activation merit further lucubrate.

The present study showed that ASId could improve cardiac functions, inhibit compensatory hypertrophy and LV remodelling, which suggests the possibility of ASId as a new therapeutic drug for the treatment of CHF.

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