Astragaloside IV Protects Heart from Ischemia and Reperfusion Injury via Energy Regulation Mechanism

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ABSTRACT

Objective: This study was designed to investigate the protective potential of AS-IV against ischemia and I/R-induced myocardial damage, with focusing on possible involvement of energy metabolism modulation in its action and the time phase in which it takes effect.

Methods: SD rats were subjected to 30 minutes LADCA occlusion, followed by reperfusion. MBF, myocardial infarct size, and cardiac function were evaluated. Myocardial structure and myocardial apoptosis were assessed by double immunofluorescence staining of F-actin and TUNEL. Content of ATP, ADP, and AMP in myocardium, cTnI level, expression of ATP5D, P-MLC2, and apoptosis-related molecules were determined.

Results: Pretreatment with AS-IV suppressed MBF decrease, myocardial cell apoptosis, and myocardial infarction induced by I/R. Moreover, ischemia and I/R both caused cardiac malfunction, decrease in the ratio of ATP/ADP and ATP/AMP, accompanying with reduction of ATP 5D protein and mRNA, and increase in P-MLC2 and serum cTnI, all of which were significantly alleviated by pretreatment with AS-IV, even early in ischemia phase for the insults that were implicated in energy metabolism.

Conclusions: AS-IV prevents I/R-induced cardiac malfunction, maintains the integrity of myocardial structure through regulating energy metabolism. The beneficial effect of AS-IV on energy metabolism initiates during the phase of ischemia.

Key words: energy metabolism, cardiac function, myocardial structure, ATP 5D

Abbreviations used: + dp/dtmax, left ventricular maximum upstroke velocity; AAR, area at risk; AS-IV, astragaloside IV; cTnI, cardiac troponin I; −dp/dtmax, left ventricular maximum descent velocity; HR, heart rate; I/R, ischemia/reperfusion; LADCA, left anterior descending coronary artery; LVDP, left ventricular diastolic pressure; LVEPD, left ventricular end diastolic pressure; LV, left ventricle; LVSP, left ventricular systolic pressure; MBF, myocardial blood flow; PCI, percutaneous coronary intervention; P-MLC2, phosphorylated myosin light chain 2; TTC, triphenyltetrazolium chloride; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling.


INTRODUCTION

Ischemic heart disease is among the top causes of death in the world [12]. PCI has currently been applied widely to deal with acute coronary syndrome, myocardial infarction, and stable angina. Although PCI can restore the blood flow in myocardium rapidly, it does not reduce the risk of serious heart events because of reperfusion injury [1,3]. Thus, strategy to prevent reperfusion injury and improve PCI outcome is currently appealing in clinic. I/R injury occurs in two phases, ischemia and reperfusion [7]. During ischemia phase, ischemic hypoxia uncouples oxidative phosphorylation from the respiratory chain, resulting in the cessation of ATP synthesis and depletion of ATP [19], which is thought to play a key role in ischemic myocardial injury. ATP deficiency causes depolymerization of F-actin [10], disarranging thin filament of cardiac myocytes. Yet, cardiac myocyte contracture initiates when the cellular ATP content decreases [8], which contributes to the cardiac malfunction. In the phase of reperfusion,
restoration of blood flow and oxygen supply provokes hypoxanthine oxidation and massive oxygen free radical production, leading to reperfusion injury. In light of the critical importance, strategies directing to intervene in energy metabolism disorder are a tempting alternative for protection of I/R-induced myocardium injury.

AS-IV (molecular structure is shown in Figure 1 [9]) is one of the components derived from a traditional herbal medicine, *Radix Astragalus*. In traditional Chinese medicine, *Radix Astragalus* has been used to deal with cardiovascular diseases for years, and thought to be able to improve energy metabolism of the heart. AS-IV was detected in the plasma of rat after given Chinese medicine which contains *Radix Astragalus* [18]. Recent studies showed that AS-IV could prevent I/R injury by inhibiting the oxidation stress and interfering with nuclear factor kappa B pathway [6,21]. However, whether AS-IV could improve the energy metabolism is not clear. This study was designed to investigate the effect of AS-IV on I/R-induced myocardial damage, with particularly focusing on the possible involvement of energy metabolism modulation in its action and the time phase over I/R challenge in which it takes effect.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats, weighing 240–260 g, were purchased from the Animal Center of Peking University (certificate no. SCXK (Jing) 2006-0008). The rats were housed in cages at temperature 22 ± 2°C, humidity 40 ± 5%, under a 12-hour light/dark cycle, and received standard diet and water *ad libitum*. The rats were fasted for 12 hours before experiment but allowed to access water freely. The investigations conformed to Guide of Peking University Animal Research Committee. Experiment protocols were approved by Peking University Biomedical Ethics Committee Experimental Animal Ethics Branch (LA2010-001).

Drug and Reagents

AS-IV was obtained from Feng Shan Jian Medicine Research Co. Ltd. (Kunming, China). It was dissolved in saline to make a solution of concentration of 1 and 10 mg/mL for different doses before experiment.

Pentobarbital sodium was purchased from Beijing Chemical Agent Ltd (Beijing, China). ELISA kits for ATP, ADP, AMP, and cTnI were from Beijing Huanya Biomedicine Technology Co. Ltd (Beijing, China). The antibodies against P-MLC2, cTnI, Bax, and Bcl-2 were bought from Cell Signaling Technology (Boston, MA, USA), the antibody against ATP 5D was from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Myocardial I/R Model and Animal Grouping

Animals were anesthetized with 2% pentobarbital sodium (60 mg/kg) by intraperitoneal injection, and placed in a supine position. A tracheal cannula was inserted via mouth, with one end being connected with an animal breathing apparatus (ALC-V8; Shanghai Alcott Biotech Co., Shanghai, China), which was set at the breathing ratio 1:1, the frequency 75 per minute, and tidal volume 12 mL/kg. A thoracotomy was performed to exposure the heart, and the LADCA was ligated with a 5/0 silk. The suture silk was released after 30 minutes, allowing reperfusion for 90 minutes. The animals in Sham and AS-IV groups underwent the same procedure but without ligation of suture silk. Ninety minutes before ischemia, the animals in AS-IV pretreatment groups were administrated through gavage with AS-IV in saline at a dose of either 1 or 10 mg/kg. The animals in Sham group and I/R-group received saline in the same way at 1 mL/kg. Three or six animals were enrolled in each group for determination of each parameter (See Table 1 for detail).

Myocardial Blood Flow

After left thoracotomy, MBF was determined at baseline, 30 minutes after ischemia, and 90 minutes after reperfusion by using a Laser-Doppler Perfusion Imager (PeriScan PIM3 System; Perimed, Stockholm, Sweden), as previously described [22]. Briefly, heart was exposed and a computer-controlled optical scanner directed a low-powered He–Ne laser beam over the exposed heart. The scanner head was positioned in parallel to the surface of heart at a distance of 18 cm. At each measuring site, the beam illuminated the tissue to a depth of 0.5 mm. A color-coded image denoting specific relative perfusion level was displayed on a video monitor, and all images were evaluated with the software LDPIwin 3.1 (PeriScan PIM3 System; Perimed). The magnitude of MBF was represented by different colors, with blue to red denoting low
to high. Results were expressed as percentages of the baseline MBF [22].

Heart Function Test
A cannulation was inserted into LV through right carotid artery, which was connected to a bio-function experiment system BL-420F (Chengdu Taimen technology Ltd, Chengdu, China). HR, LVSP, LVDP, LVEDP, +dp/dtmax, and −dp/dtmax, were evaluated at baseline, 30 minutes after ischemia, and 90 minutes after reperfusion with a BL-420F equipment [11].

Myocardial Infarct Size
At 30 minutes after ischemia and 90 minutes after reperfusion, LADCA was ligated, and 2 mL of 0.35% Evans blue (Sigma, St. Louis, MO, USA) was administrated through femoral vein. Hearts were rapidly excised and sliced into five sections (1 mm thick), parallel to the atrioventricular groove, from the apex cordis to the ligation site. Slices were incubated for 15 minutes at 37°C in a 0.375% solution of TTC (Sigma), and then photographed with a stereomicroscope connected with Digital Sight (DS-5M-U; Nikon, Nanjing, China). In so treated slices, infarction zone was stained white, AAR was pink, while noninfarction zone was blue. The myocardial area of infarct, AAR and LV was analyzed on each slice, respectively, by Image-Pro Plus 6.0 (Media Cybernetic, Bethesda, MD, USA) (n = 6). The ratios of AAR/LV (%) and infarct area/AAR (%) were calculated, and the values from five slices were averaged and used to score the degree of myocardial infarction [17].

Double Staining of F-actin and TUNEL
At 30 minutes after ischemia and 90 minutes after reperfusion, heart was perfused with saline, and then removed and fixed in 4% paraformaldehyde solution for 48 hours, processed for paraffin section (5 µm). Sections were subjected to double staining of F-actin and TUNEL. F-actin was labeled with rhodamine phalloidine (R415; Invitrogen, Carlsbad, CA, USA), and TUNEL staining was undertaken by a cell death detection kit (Roche, Basel, Switzerland), according to the manufacturer’s instruction. Then, DyLight™ 549 and DyLight™ 488-l labeled secondary antibodies were applied (KPL, Gaithersburg, MD, USA), and the nuclei were labeled with Hoechest33342. Five fields were selected from the surrounding infarction areas of the LV for each section at 40× magnification of objective, and observed with a Laser Scanning Confocal Microscope (TCS SP5; Leica, Mannheim, Germany). The number of the TUNEL-positive cells in the five fields were counted, and the average was calculated and expressed as cell number per field.

cTnI Content in Serum
Blood was collected and serum prepared using heparin as an anticoagulant at 30 minutes after ischemia and 90 minutes after reperfusion, and then samples were centrifuged for 15 minutes at 1000 g at 4°C. The supernatant was harvested, and the content of cTnI was detected using a rat cTnI ELISA Kit by microplate reader (MULTISKAN MK3; Thermo, San Jose, CA, USA) [20].

Assessment of Energy Metabolism
At 30 minutes after ischemia and 90 minutes after reperfusion, rats were perfused with saline under anesthesia, and the hearts were removed (n = 6). The tissue from LV was sampled at about 2 mm under ligature, quickly frozen in liquid nitrogen, and stored at −80°C for a maximum of one week before use. The whole protein of the tissues was extracted with a protein extraction kit (Applygen Technologies, Beijing, China), according to manufacturer’s instruction. Briefly, 80–100 mg of tissue was cut into pieces, mixed with 1 mL of RIPA containing 5 µg/mL leupeptin, 5 µg/mL aprotinin, 5 µg/mL pepstatin, and 5 mM PMSF. The mixture was homogenized, incubated on ice for 30 minutes,
and centrifuged at 19,357 g, 4°C, for 10 minutes. The myocardial content of ATP, ADP and AMP was assessed with ELISA by microplate reader (MULTISKAN MK3, Thermo), according to the manufacturer’s instructions.

**Western Blotting Assay and Real-Time PCR**

Rats were killed 30 minutes after ischemia and 90 minutes after reperfusion, and 200 mg of myocardial tissue was sampled from the surrounding of infarct area of LV, and stored at −80°C (n = 3). The whole protein was extracted as described above. The concentration of whole protein was determined with a Bio-Rad protein assay kit (Applygen Technologies), according to the manufacturer’s instruction. For each sample, the assessment was undertaken twice, taking the average as the concentration.

The whole protein was mixed with 2x electrophoresis sample buffer. After separated on 12% SDS-PAGE, the proteins were transferred to polyvinylidene difluoride membrane. After one hour blocking with 5% nonfat dry milk or 5% BSA, rinsing with TBS-Tween for three times, five minutes each, the membrane with target proteins was cut and incubated overnight at 4°C with antibodies, respectively, against P-MLC2 (1:1000), ATP5D (1:200), Bcl-2 (1:1000), Bax (1:1000), and cTnI (1:2000). And then, the membranes were rinsed three times, five minutes each, incubated with secondary antibody for one hour at room temperature, followed by rinsing with TBS-Tween three times, 10 minutes each time. The protein was quantified by scanning densitometry in the X-film using a bio-image analysis system (Image-Pro plus 6.0; Media Cybernetic). The result of each group was expressed as a relative optical density to that from Sham group.

In addition, real-time quantitative PCR was performed to detect the mRNA level of ATP 5D from each sample in accordance with the manufacturer’s protocol. RNA was extracted using RNeasy Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer’s protocol. RNA was applied for reverse transcription using a RevertAid First Strand cDNA Synthesis Kit (Fermentas Lifesciences, Vilnius, Lithuania) to generate the first strand cDNA mix. Real-time PCR was performed utilizing the ABI PRISM sequence detection system 7500 (Perkin-Elmer Applied Biosystems, CA, USA). Primer sequences (all *Rattus*): ATP5D—forward, 5′-CAGCCTG AATGCGGACTCCT-3′; reverse, 5′-GGGATTGAGATCCTAG CCCGT-3′; GAPDH—forward, 5′-AGTTCAGCCGACAG TCAAG-3′; reverse, 5′-TACTCAGCACCAGCATCACC-3′. The PCR reaction mixture (25 μL) included 2x Maxima SYBR Green/ROX qPCR Master Mix, reverse transcription product cDNA, forward and reverse primers, nuclease-free water. The reactions took place in a 96-well plate at 50°C for two minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, 58°C for one minute and plate read. All tests were performed in triplicate.

**Statistical Analysis**

All data were expressed as mean ± SE. Statistical analysis was carried out with SAS 9.3 statistical software (SAS Institute Inc., Cary, NC, USA), and one-way analysis of variance was used, followed by Tukey’s post hoc test. *p < 0.05 vs. Sham group. #p < 0.05 vs. I/R-90 group. Data are mean ± SE (n = 6).
and then for post hoc testing, Fisher’s least-significant-difference test was used for multiple comparisons between groups. For repeated measurement data, the linear mixed effects models were analyzed, and least squares means were calculated between the groups of different time points. \( p < 0.05 \) was considered as statistically significant.

**RESULTS**

**Effect of AS-IV on Myocardial Infarct Size**

The effect of AS-IV on myocardial infarct size at different doses was determined by Evans blue-TTC staining. As showing in the Figure 2A, the pink area represents ischemic myocardial tissue, the white area represents the infarction region, and the blue area represents normal myocardial tissue. We found that AS-IV was effective at the dose of 10 mg/kg, and thus chose this dose for all the experiments below. The representative heart slices in I-30 minute and I/R-90 minute groups are shown in Figure 3A and D, respectively. Obviously, myocardial tissue slices from the I-30 minute group exhibited ischemia but no infarct. In contrast, noticeable ischemia and infarct areas were observed in myocardial tissue slices in the I/R-90 minute group. As compared to the I/R-90 minute group, pretreatment with AS-IV significantly decreased the I/R-90 minutes induced myocardial infarct size, but retained a similar area of ischemic region. As shown in Figure 3B, C, E, and F, quantitative analysis of AAR/LV and infarct area/AAR confirmed the above results.
Effect of AS-IV on MBF

Figure 4A shows the color images acquired by the Laser Scanning Doppler Perfusion Imager in Sham (I/R) group, AS-IV (I/R) group, I/R-90 group and AS-IV + I/R-90 group at baseline, 30 minutes after ischemia, and 90 minutes after reperfusion. Figure 4B is the quantitative evaluation of MBF changes in the four groups, which confirmed the impression from Figure 4A.

Effect of AS-IV on Heart Function

Heart function was assessed in different conditions to evaluate the role of AS-IV in protecting heart against ischemia and reperfusion injury. As noticed in Figure 5, in comparison with Sham group, ischemia 30 minutes caused a significant decline in +dp/dtmax, and an apparent elevation in LVDP, LVEDP and −dp/dtmax, indicating an impairment on heart function. Reperfusion for 90 minutes led to a further decline in +dp/dtmax as well as a significant decrease in LVSP, and a sustained increase in −dp/dtmax, but did not
deteriorate LVDP and LVEDP. The protective role of AS-IV pretreatment for LVDP and LVEDP exhibited already at 30 minutes ischemia, but only at 90 minutes reperfusion for other parameters examined. No significant change was observed in HR in any group over the observation, nor among the groups at any time point (Figure 5A).

Effect of AS-IV on cTnI in Myocardium and Serum

As a marker of myocardial damage [2], cTnI level in myocardial tissue and serum was assessed using Western blotting and ELISA, respectively. The expression of cTnI in myocardial tissue decreased significantly 30 minutes after ischemia (Figure 6A and C) and 90 minute after reperfusion (Figure 6B and D), as compared with Sham group. In contrast, the level of cTnI in serum was very low in Sham group, but increased evidently 30 minutes after ischemia (Figure 6E), and 90 minutes after reperfusion (Figure 6F). Noticeably, the change in cTnI level in both myocardial tissue and serum after ischemia and I/R was significantly attenuated by pretreatment with AS-IV (Figure 6C).

Effect of AS-IV on Energy Metabolism

To address the energy metabolism in different conditions, the ratio of ATP/ADP and ATP/AMP in cardiac tissue was explored. As shown in Figure 7A–D, AS-IV alone had no effect on either ATP/ADP or ATP/AMP compared with Sham group. Notably, ATP/ADP and ATP/AMP decreased dramatically at 30 minutes after ischemia, and remained at low level by I/R-90 minutes, indicating a more catabolism of ATP. However, pretreatment with AS-IV significantly prevented ATP/ADP and ATP/AMP from reduction both at 30 minutes after ischemia and 90 minutes after reperfusion.

We next determined the expression of ATP 5D and P-MLC2 in myocardial tissue. As a subunit of ATP synthase, the expression of protein and mRNA of ATP 5D had an obvious reduction in response to I-30 minutes and I/
R-90 minutes (Figure 8A2, A3, B2, and B3). The level of P-MLC2 increased prominently in response to 30 minutes ischemia (Figure 8A4) and I/R-90 minutes challenge (Figure 8B4). Of notice, pretreatment with AS-IV prevented all the alterations evoked by ischemia and I/R.

Effect of AS-IV on Cardiac Structure and Myocardial Cell Apoptosis
To gain insight into the effect of AS-IV on the alteration in myocardium structure and apoptosis, double staining of F-actin and TUNEL was carried out for the surrounding infarction areas of the LV myocardial tissue from various groups. The representative images are displayed in Figure 9A and B, wherein nuclei were stained blue, F-actin red, and TUNEL-positive cells green. At 30 minutes after ischemia, myocardial tissue became injury with disrupted myocardial fibers and few apoptotic cells, while this injury was protected against by pretreatment with AS-IV. At 90 minutes after reperfusion, the myocardial tissue displayed more distinct alterations compared with Sham group, exhibiting rupture of myocardial fibers, degradation of F-actin, and numerous TUNEL-positive cells. These changes were all alleviated by pretreatment with AS-IV.

Effect of AS-IV on the Expression of Apoptosis-Related Proteins
Bcl-2 and Bax have been well accepted as apoptosis regulated proteins, with Bcl-2 acting as an anti-apoptosis factor, and Bax as a pro-apoptosis molecule [13]. Therefore, we investigated the expression of Bcl-2 and Bax by Western blotting, and calculated the ratio of Bax/Bcl-2. As shown in Figure 10, the expression of Bcl-2 and Bax had no obvious change among the groups at 30 minutes after ischemia, while at 90 minutes after reperfusion the ratio of Bax/Bcl-2 significantly increased as compared to Sham group, and such upregulation was suppressed by pretreatment with AS-IV.

DISCUSSION
This study showed that I/R-induced myocardium injury manifested differently in ischemia and reperfusion phase. In ischemia phase, the injury presented as a decrease in MBF, ATP/ADP, ATP/AMP, the expression of ATP 5D and the content of cTnI in myocardium, and an increase in phosphorylation of MLC2 and the content of cTnI in serum, accompanying with disrupted F-actin; while in reperfusion phase, MBF remained deceased compared to baseline implying a no-reflow due to the impairment of microvasculature, and apoptosis and infarction occurred in addition to the insults observed in ischemia phase. Interestingly, pretreatment with AS-IV attenuated I/R-elicited insults in myocardium in both ischemia and reperfusion phase, suggesting it as a potential option for protecting heart from I/R injury.

As expected, most of ischemia-evoked insults are associated with disorder of energy metabolism. Energy metabolism disorder in ischemia phase was documented in the present case by the reduction in ATP/ADP and ATP/AMP. F-actin integrity in myocardium was found disrupted in this phase, an outcome that is known to depend on ATP availability.
LVEDP and LVDP elevated significantly at 30 minutes ischemia, indicative of a so-called ischemic contracture, which is related to ATP depletion [15], since a too low ATP availability leads to cross-bridges remaining trapped in a rigor state [14]. Ischemia led to an increase in phosphorylation of MLC2 in this study. MLC2 phosphorylation are mediated by myosin light chain kinase as well as by protein kinase C, both of which are Ca2+ dependent, and ATP depletion caused cytoplasmic Ca2+ overload may be expected to increase MLC2 phosphorylation [16]. Of notice, ATP 5D expression decreased in ischemia phase. As one of the subunits of ATP synthase, ATP 5D plays an important part in ATP synthesis [4]. The result of this study implies that reduced ATP 5D expression along with hypoxia contributes to the observed ATP depletion. Importantly, AS-IV pretreatment protected against energy metabolism disorder and the resultant insults, suggesting the beneficial role of AS-IV initiated early in the ischemia phase. Moreover, the results

Figure 8. The effect of AS-IV on the expression of ATP 5D and P-MLC2 in myocardium of rats. (A) The expression of ATP 5D and P-MLC2 at 30 minutes after ischemia in various groups. Shown are representative Western blotting bands (A1), semi-quantitative analysis of ATP 5D (A2) and P-MLC2 (A4) protein, and the mRNA level of ATP 5D (A3), respectively. *p < 0.05 vs. Sham (I) group, #p < 0.05 vs. I-30 group. (B) The expression of ATP 5D and P-MLC2 at 90 minutes after reperfusion in various groups. Shown are the representative Western blotting bands (B1), semi-quantitative analysis of ATP 5D (B2) and P-MLC2 (B4) protein, and mRNA level of ATP 5D (B3), respectively. *p < 0.05 vs. Sham (I/R) group, #p < 0.05 vs. I/R-90 group. Values are means ± SE (n = 3).
that AS-IV-attenuated MBF and ATP 5D expression suggested that the protective role of AS-IV in energy metabolism is attributable to increased oxygen supply as well as to enhanced ATP synthesis capacity. Nonetheless, the detailed mechanism responsible for the beneficial role of AS-IV in energy metabolism needs further study.

**Figure 9.** The effect of AS-IV on myocardial apoptosis and F-actin. (A) and (B): Presented are the representative photographs of double staining of F-actin and TUNEL. Nuclei are stained with blue, F-actin red, and TUNEL-positive cells green (arrow). Bar = 25 μm. (C) and (D): Quantitative analysis of apoptosis cells among the various groups. Ordinates are cell number per field. *p < 0.05 vs. Sham (I/R) group, #p < 0.05 vs. I/R-90 group. Values are means ± SE (n = 3).

**Figure 10.** The effect of AS-IV on the expression of Bcl-2 and Bax in myocardium of rats. (A) and (B) The representative Western blotting bands of Bcl-2 and Bax in myocardium at 30 minutes after ischemia (A) and 90 minutes after reperfusion (B), respectively. (C) and (D) The semi-quantitative analysis of Bax/Bcl-2 at 30 minutes after ischemia (C) and 90 minutes after reperfusion (D), respectively. *p < 0.05 vs. Sham (I/R) group, †p < 0.05 vs. I/R-90 group. Values are means ± SE (n = 3).
AS-IV is widely used to cope with various diseases, including acute kidney injury [6], Parkinson’s disease [21], and diabetic nephropathy [5]. Several studies reported that AS-IV exerted its multiple action through inhibiting oxidative stress, interfering in NF-kappa B-mediated inflammatory process, and Bax-mediated apoptosis pathways [5,21]. In a myocardial I/R injury model in this study, the anti-apoptosis potential of AS-IV was further documented, as shown by the reduction of TUNEL-positive cells after AS-IV pretreatment. Pretreatment with AS-IV significantly prevented the increase in Bax/Bcl-2 ratio after I/R, suggesting that the anti-apoptosis effect of AS-IV was related to the suppression of Bax-mediated pathway. AS-IV protection of cardiac myocytes from apoptosis ultimately led to the attenuation of myocardial injury, as evidenced by the reduction in myocardial infarct size. Of notice, I/R-induced cardiac myocyte apoptosis and myocardium infarction emerged at 90 minutes after reperfusion but not at 30 minutes after ischemia, showing that these impairments are reperfusion injury in nature. On the other hand, an increase in serum cTnI level and a decrease of cTnI content in heart tissue were observed already at 30 minutes after ischemia, indicating that cardiac myocyte injury took place in this phase in a form of nonapoptosis. AS-IV prevented ischemia induced increase in serum cTnI level, implying that AS-IV protects cardiac myocyte from ischemia injury via a mechanism other than depressing apoptosis. This mechanism is most likely a modulation of energy metabolism.

In conclusion, AS-IV pretreatment protected against myocardium injury and cardiac malfunction after I/R, which may be related to its potential to restore the energy metabolism disorder occurred in ischemia phase. The results provide support for AS-IV as a novel therapeutic approach to protect against I/R-induced myocardial injury. In addition, the results of this study opened an avenue for development of new drug to cope with cardiac I/R injury by targeting energy metabolism.

**REFERENCES**


