European Review for Medical and Pharmacological Sciences 2015; 19: 759-766

Protective effects of valsartan and benazepril combined with atorvastatin on cardiorenal syndrome in rats

S.-Y. TANG, D.-F. PENG, Y.-J. HU, J. CHEN

Department of Cardiology, Affiliated Puai Hospital, Tongji Medical College, Wuhan, Hubei Province, China

Abstract. – OBJECTIVE: To study the protective effects of valsartan (Val) and benazepril, (Ben) combined with atorvastatin (Ato), on cardiorenal syndrome (CRS) in rats.

MATERIALS AND METHODS: After establishing cardiorenal syndrome model, the rats were randomly divided into control, Ato, Ben+Ato and Val+Ato groups, which were treated with corresponding drugs. Before and 4 weeks after treatment, the serum creatinine (Scr), blood urea nitrogen (BUN), type-B natriuretic peptide (BNP), aldosterone (ALD), angiotensin (Ang) II, C-reactive protein (CRP), blood lipid and urine protein were determined. The left ventricular systolic pressure (LVSP), left ventricular diastolic pressure (LVDP), left ventricular end-diastolic pressure (LVEDP) as well as maximum rising and falling rates of left ventricular pressure (±dp/dt-max) were detected. The heart weight index was also determined.

RESULTS: 6, 3, 1 and 2 rats control, Ato, Ben+Ato and Val+Ato groups died, respectively. Compared with control group, the serum Cr, BUN, BNP, ALD, CRP and urinary protein levels in treatment groups significantly decreased, and the blood lipid level, LVDP, LVEDP and heart weight index significantly decreased, with increased LVSP. No statistically significant difference was observed among treatment groups.

CONCLUSIONS: Valsartan and benazepril, combined with atorvastatin, can have significant protective effects on cardiorenal functions of rats with CRS, with no significant difference between these two drugs.

Key Words:
Alsartan, Benazepril, Atorvastatin, Cardiorenal syndrome, Rat.

Introduction

Patients with cardiorenal syndrome (CRS) experience poor prognosis. Clinical focus on CRS has increased recently. The possible pathogenesis of CRS may be haemodynamic changes, endothelial dysfunction, inflammation and renin-angiotensin-aldosterone system (RAAS) and/or sympathetic system activation. Changes in any of the aforementioned risk factors will result in a cascade reaction of the other factors, thus, leading to a vicious cycle and causing damage to cardiorenal structure and functions[1]. During an international consensus conference of Acute Dialysis Quality Initiative, CRS was divided into five types. Type II CRS refers to a chronic kidney disease caused by a chronic heart dysfunction, whereas type IV CRS refers to a cardiac dysfunction caused by a chronic kidney disease[2]. However, the exact mechanism that causes and maintains cardiorenal interaction remains unclear. By considering chronic CRS pathogenesis, our research team has established a rat model for CRS, which is induced by 3/4 kidney resection combined with subcutaneous ISO injection. In this model, inflammation and RAAS activation can promote the occurrence and development of cardiorenal failure[3]. The process of determining the targeted therapy under the pathophysiological mechanism is important. Angiotensin-converting enzyme inhibitor (ACEI) and adrenergic receptor binder (ARB) are RAAS inhibitors, whereas statins have anti-inflammatory aside from lipid-lowering effects. At present, comparisons between ACEI and ARB effects, when combined with statins for treating CRS, remain inadequate. The difference in protective effects of benazepril and valsartan combined with atorvastatin towards the cardiorenal functions of rats with CRS were researched in the present study.

Materials and Methods

Animals

One hundred male SD rats, approximately 180±20 g, were provided by the Experimental

Corresponding Author: Dingfeng Peng, MD; e-mail: dingfengpeng@126.com

Animal Center, Tongji Medical College, Huazhong University of Science. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Affiliated Puai Hospital, Tongji Medical College.

Grouping and Modeling

The rats were kept in the laboratory according to the preparative method of the cardiorenal model[2]. After one week, the rats were anesthetised with 10% chloral hydrate (0.3 mL/100 g, intraperitoneal injection), and 1/2 tissue of the lower pole of the left kidney was removed. One week later, the right kidney was totally excised. After one week of conventional breeding, ISO (100 mg/kg, twice at an interval of 24 h; Sigma-Aldrich Corporation, Saint Louis, MO, USA) was injected subcutaneously. The diet, fur colour, activity, cyanosis and oedema of the rats were observed. After four weeks of normal drinking and diet, the surviving rats were kept in separate metabolic cages, and then, 24 h urine was collected. Coomassie brilliant blue G-250 dye (Bradford method; Wuhan Jiyinmei Biotechnology Co. Ltd., Wuhan, China) was applied to determine urinary protein content and calculate 24 h urinary protein excretion. Subsequently, a serum of rat ocular venous blood was prepared for determining serum creatinine (Scr), blood urea nitrogen (BUN), type-B natriuretic peptide (BNP), aldosterone (ALD), angiotensin (Ang) II, C-reactive protein (CRP) level and blood lipid level (ELISA method; Wuhan Jiyinmei Biotechnology Co. Ltd., Wuhan, China). The rats identified with cardiorenal syndrome were randomly divided into the control, atorvastatin (Ato), benazepril combined with atorvastatin (Ben+Ato) and valsartan combined with atorvastatin (Val+Ato) groups, with 15 rats in each group.

Drug Intervention

The treatment groups were given with atorvastatin (10 mg/kg·d; Pfizer Inc., New York, NY, USA), benazepril (10 mg/kg·d; Novartis Inc., Basel, Switzerland) combined with atorvastatin (10 mg/kg·d; Pfizer Inc., New York, NY, USA), and valsartan (80 mg/kg·d; Novartis Inc., Basel, Switzerland) and atorvastatin (10 mg/kg·d; Pfizer Inc., New York, NY, USA), respectively. The control group was given with normal saline. Treatment was administered daily. After four weeks, the previously mentioned serum markers and 24 h urinary protein were retested.

Determining Left Ventricular Function and Haemodynamic Parameters

Twenty-four hours after blood sampling, left ventricular cannulation was performed through the left common carotid artery for detecting left ventricular functions. The operation method was as follows. An incision was made along the neck midline, and then, the right carotid artery was exposed to perform blunt dissection. The left ventricular catheter, prefilled with 10% heparinised saline, was retrograde catheterized into the left ventricle through the right common carotid artery. The other end of the catheter was connected to the pressure transducer and provided input into the BL-420F biofunctional experiment system (Chengdu Taimeng Technology Co., Ltd., Chengdu, China) for haemodynamic tests, i.e., left ventricular systolic pressure (LVSP), left ventricular diastolic pressure (LVDP), left ventricular end-diastolic pressure (LVEDP) and falling rates of left ventricular pressure (±dp/dt-max).

Determining Heart Weight Index

After the aforementioned cardiac functions were detected, the heart was removed and rinsed with saline. The excess water was absorbed. The left ventricle was removed and weighed (LVW). The heart weight index was calculated based on body weight (BW), and LVW/BW was used to judge the degree of left ventricular remodeling.

Picric Acid-Sirius Red Staining

The left ventricular tissue was treated with 4% neutral formalin (10 times the amount of the tissue) for fixation. The tissue was then embedded in paraffin and cut into 4 μm thick slices. After picric acid-sirius red staining, the same vision field of slices was observed under microscope (100 ×). The volume fraction of myocardial collagen fiber was calculated using Image-Pro Plus 6.0 software.

Statistical Analysis

Data were expressed as mean ± standard deviation ($x \pm s$). Inter-group average data were analysed using χ^2 test and ANOVA. All calculations were performed using SPSS 17.0 statistical software (IBM Corporation, New York, NY, USA), with $p < 0.05$ significant difference.

Results

General Characteristics and Survival Rate

After four weeks, the rats in the control group ate less and their body weights increased slowly compared with those in the treatment groups. The rats acted slowly; the mouth and nose exhibited cyanosis; their respiration had an evident wheeze; their paws had oedema; their daily activity was reduced and their grab response became poor. Six rats in the control group, three rats in the Ato group, one rat in the Ben+Ato group and two rats in the Val+Ato group died. Due to small sample size, the treatment groups were combined, and the mortality rate of combined group had significant difference with control group ($p < 0.05$).

Comparison of Blood Lipid Levels

The comparison of serum total cholesterol (TC), triglyceride (TG) and low-density lipoprotein (LDL) between the control group and the treatment groups exhibited no statistically significant difference ($p > 0.05$) before the treatment (Figure 1A). Four weeks after the treatment, serum TC, TG and LDL levels in the treatment groups were significantly lower ($p < 0.05$) compared with those in the control group. Meanwhile, the aforementioned lipid levels exhibited no statistically significant difference ($p > 0.05$) between the Ato, Ben+Ato and Val+Ato groups (Figure 1B).

Comparison of Cardiorenal Function Parameters

Serum Cr, BUN, BNP, ALD, Ang II, CRP and 24 h urine protein in the control group exhibited no statistically significant difference with those in the treatment groups (Table I). Four weeks after the treatment, serum Cr, BUN, BNP, ALD, CRP and 24 h urine protein levels were significantly lower in the treatment groups than in the control group. Compared with Ato group, the efficacies in Ben+Ato and Val+Ato group were more obvious, with no significant difference between the later two groups. Moreover, the serum Ang II level in Ben+Ato group was significantly decreased compared with Val+Ato group (Table II).

Four weeks after the treatment, the left ventricular functions and haemodynamic parameters of each group were evaluated. In control group, the LVSP decreased, and the LVDP and LVEDP significantly increased. The maximum diastolic velocity (-dp/dtmax) decreased. The treat groups had improved heart function, with significant difference with control group. Compared with Ato

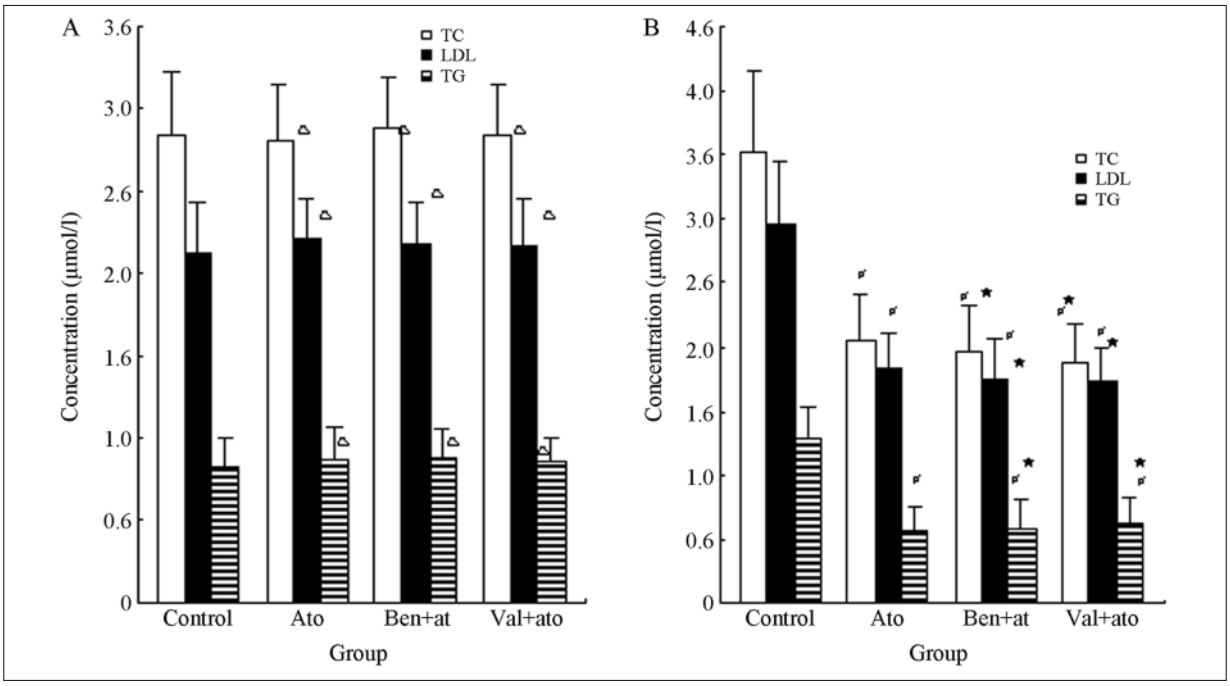

Figure 1. Serum TC, TG, LDL before and after treatment. ***A,*** Serum TC TG LDL before treatment. ***B,*** Serum TC TG LDL after treatment. Note: Compared with control group, $^{\Delta}p > 0.05$; Compared with control group, $^{\#}p < 0.01$; compared with Ato group, $^{\star}p > 0.05$.

Table I. Concentrations of Scr, BUN, BNP, ALD, Ang II, CRP and urine protein.

Group	Scr (μmol/l)	BUN (mmol/l)	BNP (pg/ml)	ALD (pg/ml)	Ang II (pg/ml)	CRP (μg/l)	Urine-protein (mg/24h)
Control	77.5 ± 8.7	10.2 ± 1.5	56.5 ± 4.6	137.6 ± 16.1	816.3 ± 57.2	568.5 ± 42.1	70.5 ± 12.7
Ato	75.8 ± 8.5$^{\Delta}$	10.0 ± 1.6$^{\Delta}$	56.1 ± 4.3$^{\Delta}$	135.3 ± 16.5$^{\Delta}$	823.2 ± 76.1$^{\Delta}$	580.5 ± 43.2$^{\Delta}$	72.6 ± 12.5$^{\Delta}$
Ben+Ato	75.3 ± 8.9$^{\Delta}$	10.4 ± 1.6$^{\Delta}$	56.0 ± 4.8$^{\Delta}$	136.3 ± 15.5$^{\Delta}$	827.8 ± 73.4$^{\Delta}$	576.5 ± 45.2$^{\Delta}$	72.0 ± 13.5$^{\Delta}$
Val+Ato	77.8 ± 9.3$^{\Delta}$	10.3 ± 1.8$^{\Delta}$*	57.0 ± 5.3$^{\Delta}$	134.6 ± 13.8$^{\Delta}$	835.3 ± 68.9$^{\Delta}$	565.3 ± 45.6$^{\Delta}$	72.0 ± 15.3$^{\Delta}$

Note: Compared with Control group, $^{\Delta}p > 0.05$.

Table II. Concentrations of Scr, BUN, BNP, ALD, Ang II, CRP and urine protein 4 weeks after the treatemnt.

Group	Scr (μmol/l)	BUN (mmol/l)	BNP (pg/ml)	ALD (pg/ml)	Ang II (pg/ml)	CRP (μg/l)	Urine-protein (mg/24h)
Control	89.8 ± 9.5	12.2 ± 2.5	89.5 ± 6.9	230.5 ± 30.5	1023.2 ± 86.4	702.8 ± 63.2	102.5 ± 16.3
Ato	75.6 ± 9.3**	10.0 ± 2.3	71.6 ± 9.6***	180.3 ± 50.8*	1005.8 ± 108.3	546.3 ± 40.9***	80.6 ± 15.6**
Ben+Ato	63.8 ± 8.6***,#	7.2 ± 2.9***,#	48.9 ± 10.2***,###	145.8 ± 42.3***	838.3 ± 205.2*,#	450.9 ± 38.5***,###	64.6 ± 13.4***,#
Val+Ato	60.6 ± 9.8***,##,$^{\Delta}$	7.6 ± 2.0***,#,$^{\Delta}$	46.5 ± 6.5***,###,$^{\Delta}$	140.7 ± 45.2***,#,$^{\Delta}$	1106 ± 102.9	460.9 ± 36.5***,###,$^{\Delta}$,	62.1 ± 10.3***,##,$^{\Delta}$

Note: Compared with control group, $^{*}p < 0.05$, $^{***}p < 0.001$; compared with Ato group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$; compared with Ben+Ato group, $^{\Delta}p > 0.05$.

group, the efficacies in Ben+Ato and Val+Ato groups were more significant, but there was no significant difference between Ben+Ato and Val+Ato groups (Table III).

Comparison of Body Weight, Left Ventricular Weight and Left Ventricular Weight Index

Four weeks after the treatment, the rats in the control group were significantly lighter, whereas their left ventricular weight increased. The left ventricular weight index (LVW/BW) in control group was 2.81±0.15, and that in Ato group was 2.48±0.32, which was significantly decreased ($p < 0.01$). The left ventricular weight indexes in Ben+Ato and Val+Ato groups were 1.50±0.32 and 1.59±0.26, respectively, which were significantly lower than control group and Ato group, respectively ($p < 0.001$). The difference between the Val+Ato and Ben+Ato groups was not statistically significant ($p > 0.05$) (Table IV).

Picric Acid-Sirius Red Staining Results

In picric acid-sirius red staining, the collagen fiber presented red and myocardial cells presented yellow. One left ventricle cross-section with complete form and uniform staining was selected from each section. The section was divided into 4

Table III. Comparison of left ventricular function and hemodynamic parameters among four groups.

Group	LVSP (mmHg)	LVDP (mm Hg)	LVEDP (mm Hg)	dp/dtmax (mmHg/s)	-dp/dtmax (mm Hg/s)
Control	96.5 ± 10.2	4.2 ± 1.9	8.6 ± 2.2	4358 ± 732	-3500 ± 870
Ato	110.6 ± 13.6*	2.3 ± 1.8*	5.6 ± 2.0*	5500 ± 965*	-4500 ± 968*
Ben+Ato	128.8 ± 12.3***,###	1.2 ± 1.6***	2.3 ± 2.2***,###	6500 ± 865***,#	-5320 ± 785***,#
Val+Ato	130 ± 18.9***,###,$^{\Delta}$	1.3 ± 1.2***,$^{\Delta}$	2.8 ± 1.6***,###,$^{\Delta}$	6598 ± 436***,###,$^{\Delta}$	-5600 ± 830***,##,$^{\Delta}$

Note: Compared with control group, $^{*}p < 0.05$, $^{***}p < 0.001$; compared with Ato group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$; compared with Ben+Ato group, $^{\Delta}p > 0.05$.

Table IV. Comparison of LVW/BW among four groups.

Group	LVW (g)	BW (kg)	LVW/BW (g/kg)
Control	0.68 ± 0.06	0.26 ± 0.04	2.81 ± 0.15
Ato	0.61 ± 0.03	0.30 ± 0.03	2.48 ± 0.32**
Ben+Ato	0.50 ± 0.04	0.34 ± 0.03	1.50 ± 0.33***,##
Val+Ato	0.49 ± 0.05	0.33 ± 0.02	1.59 ± 0.26***,##,Δ

Note: Compared with control group, $^{**}p < 0.01$, $^{***}p < 0.001$; Compared with Ato group, $^{\#\#\#}p < 0.001$; compared with Ben+Ato group, $^{\Delta}p > 0.05$.

quadrants, and 4 vision fields (2 epicardial vision fields and 2 endocardial vision fields) were randomly selected from each quadrant. The ratio of collagen fiber area to total vision field area was calculated. The sections were observed after staining. In the control group, cardiac hypertrophy was significant; myocardial cells thickened with an uneven arrangement and myocardial fibrosis was more pronounced. By contrast, improvement was significant in the treatment groups. The volume fraction of myocardial collagen fiber in control group was 29.3±2.7%, and that in Ato, Ben+Ato and Val+Ato groups was 19.8±3.4%, 10.8±2.1% and 11.6±1.9%, respectively, which was significantly lower than control group ($p < 0.001$). Compared with Ato group, the myocardial fibrosis in Ben+Ato and Val+Ato groups were also significantly mitigated ($p < 0.001$), with no significant difference between them ($p > 0.05$) (Figure 2).

Discussion

At present, no special treatment for actively correcting heart and renal failure among CRS patients is available. The protective effects of ARB and ACEI against renal damage are clearly evident, The guidelines of several chronic heart failure treatments recommend ACEI as the basis for treating chronic heart failure. In recent years, however, the CHARM test results, has improved the position of ARB drugs for treating heart failure. The evidence for candesartan and valsartan in reducing mortality and morbidity rates is clear[4,5]. The JIKEI HEART trial considered that the Asian population can benefit more from valsartan-based treatment programs[6]. However, neither ACEI nor ARB can completely inhibit (RAS) activation individually. Aside from RAS activation, CRS progress also involves inflammation and oxidative stress. Therefore, the addition of a full blocking RAS system, endothelial function improvement and oxidative stress and vascular inflammatory reaction reduction are necessary for further improving prognosis. If the RAS system is blocked, the endothelial function is improved and oxidative stress and vascular inflammation are reduced, then the prognosis can be improved. In addition to lipid regulation effects, statins have pleiotropic effects such as anti-inflammatory, antioxidant, vascular endothelial function protection and neurohumoral factor regulation. *In vitro* studies and retrospective analyses indicate that according to basic therapy, the use of statins can reduce or delay the onset and progression of chronic heart failure[7]; however, the results of several prospective clinical studies

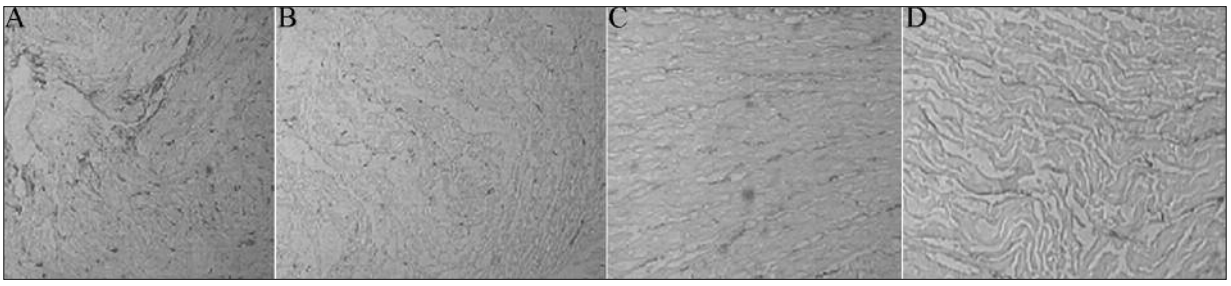

Figure 2. Picric acid-sirius red staining of cardiac muscular tissue in the left ventricle (magnification, ×100). ***A,*** Control group. ***B,*** Ato group; C Ben+Ato group; D Val+Ato group.

do not support this view[8]. Meanwhile, an increasing number of evidence show that patients with renal dysfunction can also benefit significantly from the early stage of statin therapy[9,10].

Clinical observations of statins and ACEI/ARB combination among patients with heart failure considered that such treatment can further improve ventricular remodeling and further decline inflammatory factors, such as CRP. Moreover, the combination of statins and losartan medicines has synergistic protective effects towards the vessels[11,12], thus, probably leading to better results. However, large-scale clinical observations and comparative studies are still lacking. The objective of the present study is to explore the differences in the protective effects of ACEI and ARB towards rats with CRS when these drugs are combined with statins.

During the present research, the CRS model was induced by 3/4 nephrectomy and subcutaneous ISO injection. The BNP, ALD, Ang II and CRP of the rats with CRS increased significantly, and the mechanism depended on the fact that activating the angiotensin system led to increased Ang II release. This process resulted in the vasoconstriction and contraction of renal efferent arteries, increase in cardiac remodeling and release of aldosterone. Consequently, sodium retention and myocardial fibrosis promotion were enhanced, thus leading to cardiorenal dysfunction. Picric acid-sirius red staining exhibited myocardial hypertrophy, myocardial fibrosis and necrosis as well as CRS progression. Myocardial remodeling will gradually increase, thus resulting in a broken compensation period for heart functions[3].

Proteinuria is a powerful independent risk factor of cardiovascular diseases. Once massive proteinuria appears, the process of atherosclerosis will be accelerated, endothelial dysfunction will appear and risks of end-organ damage will increase, thus leading to serious cardiovascular events and death[13]. ACEI and ARB exhibit pronounced vasodilation towards glomerular efferent arterioles. These drugs can increase renal blood flow as well as reduce renal vascular resistance and glomerular capillary pressure. However, these drugs do not affect or only slightly reduce glomerular filtration rate, improve and prevent renal function deterioration as well as reduce urinary protein. BNP is a recently discovered polypeptide with diagnostic value for heart failure. One study[14] showed that the plasma BNP level of heart failure patients with renal impairment is positively correlated with the degree of heart disease, and can help in determining prognosis. In the present study, serum Cr and BUN of the Ato, Ben+Ato and Val+Ato groups decreased after the treatment compared with those in the control group. Left ventricular function improved; left ventricular weight index decreased and myocardial fibrosis improved, thus indicating that heart and kidney functions improved. Simultaneously, BNP and 24 h urinary protein decreased significantly. This indicated that, compared with control and Ato groups, Ben+Ato and Val+Ato groups were able to improve prognosis during cardiorenal protection, with no significant difference between them.

Although clinical research results on the use of statins for treating patients with heart failure differed, a recent meta-analysis involving 10 clinical trials showed that statins can significantly improve ejection fraction and decrease rehospitalisation rate[15]. Similarities are found between atherosclerosis and glomerular sclerosis; thus, the beneficial effects of statins on cardiovascular diseases can also be applied to the kidneys. A number of random studies showed that long-term statin therapy can significantly improve renal function[16,17]. In the present experiment, the lipid levels of the treatment group significantly decreased, thus, indicating that atorvastatin has a protective effect towards cardiorenal function by lowering lipid levels. Moreover, the decline in serum CRP level in the treatment groups showed that statins has other effects aside from lipid regulation, such as increasing NO synthase and inhibiting endothelial cells, macrophages adhesion molecules, inflammatory cytokines, growth factors, etc[18-20]. Regarding the current controversy on statin therapy for heart failure patients, a number of experts believe that the therapeutic effect of statins is positive towards patients with elevated CRP levels[21]. Our results indicated that statin therapeutic effect was positive towards CRS patients with elevated CRP.

In the present study, serum Ang II level in the Ben+Ato group significantly decreased compared with that in the control group, whereas serum Ang II levels were minimally elevated in the Val+Ato group, with a statistically significant difference between the two groups. This result indicates that the protection mechanisms of the two treatments are different towards CRS. Peripheral serum Ang II level in the valsartan group increased, thus, indicating that valsartan primarily acted on the local organ to block RAAS. Local RAS has an important function in the structural

remodeling of the heart and kidneys. ARB drugs can effectively lower blood pressure by blocking the RAS system, and can effectively reverse tissue remodeling by blocking the local RAS, thus, particularly inhibiting excessively produced local Ang II. Therefore, compared with other antihypertensive drugs, ARB has unique protective effects that go beyond decompression towards important target organs such as the heart and the kidneys. ARBs and statins can reduce myocardial apoptosis, which is relevant in inhibiting cardiac remodeling[12]. Fluvastatin can reduce the protein expression of AT1-R mRNA and AT1-R of cocultured vascular smooth muscle cells[22], thus demonstrating that statins can act through the interference of the AT1-R expression. The combination of statins and ARB can further improve ventricular remodeling and further induce inflammatory factors such as CRP. In CRS, blood circulation and RAS in regional myocardial and renal tissues are activated. Ang II has an important function in these processes. Statins can decrease AT1-R expression, whereas ARB can further block AT1-R, thus preventing myocardial fibrosis, Ang II promotion, vascular and ventricular remodeling as well as aldosterone synthesis and secretion reduction.

Serum Ang II levels in the Ben+Ato group significantly decreased, thus, indicating that ACEI inhibited the angiotensin-converting enzyme and reduced Ang II formation; thereby reducing Ang II activities, such as vascular contraction effects as well as the release of aldosterone, endothelin and other vasoactive substances. Consequently, cardiac and vascular hypertrophies are prevented and reversed, and heart weight and wall thickness are reduced. These effects are beneficial to heart failure and to reconstruction relief and prevention. ACEI can also increase bradykinin concentration as well as nitric oxide and prostacyclin generation through relevant mechanisms. Consequently, blood vessels are relaxed, and antiplatelet aggregation as well as cardiovascular cell hypertrophy and hyperplasia are exhibited. ACEI also has antioxidant and free-radical scavenging effects, which are associated with the use of statins. These effects can be beneficial to vascular protection[23] and cardiorenal protection enhancement. Although the cardiorenal protection mechanisms of ACEI and ARB are different when they are combined with statins, the present study shows that no significant difference is found between the two drugs in CRS treatment when these drugs are combined with statins.

Conclusions

The combination of ACEI/ARB with statins can provide stronger cardiorenal protection. The mechanisms probably result from the synergies of statins with ACEI or ARB, which can enhance the inhibitory effect against RAS, with lipid-lowering and pleiotropic effects. During cardiorenal protection, the CRS process can also be delayed. Therefore, patients in the early stage of heart failure or impaired renal function should be treated immediately with statins to suppress the onset of CRS development more effectively.

Acknowledgements

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH).

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Bongartz LG, Braam B, Verhaar MC, Cramer MJ, Goldschmeding R, Gaillard CA, Doevendans PA, Joles JA. Transient nitric oxide reduction induces permanent cardiac systolic dysfunction and worsens kidney damage in rats with chronic kidney disease. Am J Physiol Regul Integr Comp Physiol 2010; 298: R815-23.

2) Ronco C, McCullough P, Anker SD, Anand I, Aspromonte N, Bagshaw SM, Bellomo R, Berl T, Bobek I, Cruz DN, Daliento L, Davenport A, Haapio M, Hillege H, House AA, Katz N, Maisel A, Mankad S, Zanco P, Mebazaa A, Palazzuoli A, Ronco F, Shaw A, Sheinfeld G, Soni S, Vescovo G, Zamperetti N, Ponikowski P. Cardio-renal syndromes: report from he consensus conference of the acute dialysis quality initiative. Eur Heart J 2010; 31: 703-711.

3) Peng DF, Tang SY, Hu YJ, Chen J, Yang L. Pathophysiological model of chronic heart failure complicated with renal failure caused by three-quarter nephrectomy and subcutaneous injection of isoprenaline. Exp Ther Med 2013; 5: 835-839.

4) Granger CB, McMurray JJV, Yusuf S, Held P, Michelson EL, Olofsson B, Ostergren J, Pfeffer MA, Swedberg K. Effects of candesartan in patients with chronic heart failure and reduced left-ventricularsystolic function intolerant to angioten sinc-onverting-enzyme inhibitors: the CHARM-alternative Trial. Lancet 2003; 362: 772-776.

5) Abraham I, MacDonald K, Hermans C, Aerts A, Lee C, Brié H, Vancayzeele S. Real-world effectiveness

of valsartan on hypertension and total cardiovascular risk: review and implications of a translateonal research program. Vasc Health Risk Manag 2011; 7: 209-235.

6) Jun S, Takahisa S, Masahiro K, Yamada H, Matsubara H. Cardio-cerebrovascular protective effects of valsartan in high-risk hypertensive patients with coronary artery disease (from the Kyoto Heart Study). Am J Cardiol 2012; 109: 1308-1314.

7) Ramasubbu K, Estep J, White DL, Deswal A, Mann DL. Experimental and clinical basis for the us of statins in patients with ischemic and nonischemic cardiomyopathy. J Am Coll Cardiol 2008; 51: 415-426.

8) GISSI-HF Investigators, Tavazzi L, Maggioni AP, Marchioli R, Barlera S, Franzosi MG, Latini R, Lucci D, Nicolosi GL, Porcu M, Tognoni G. Effect of rosuvastatin in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. Lancet 2008; 372: 1231-1239.

9) Strippoli GF, Navaneethan SD, Johnson DW, Perkovic V, Pellegrini F, Nicolucci A, Craig JC. Effects of statins in patients with chronic kidney disease: meta-analysis and meta-regression of randomised controlled trials. Br Med J 2008; 336: 645-651.

10) Fujii M, Inoguchi T, Maeda Y, Sasaki S, Sawada F, Saito R, Kobayashi K, Sumimoto H, Takayanagi R. Pitavastatin ameliorates albuminuria and renal mesangial expansion by downregulating NOX4 in db/db mice. Kidney Int 2007; 4: 473-480.

11) Horiuchi M, Cui TX, Li Z, Li JM, Nakagami H, Iwai M. Fluvastatin enhances the inhibitory effects of a selective angiotensin II type1 receptor blocker, valsartan, on vascularneointimal formation. Circulation 2003; 107: 106-112.

12) Bayorh MA, Layas MF, Mann G, Feuerstein GZ, Eatman D. The effect of diet on simvastatin and losartan enhancement endothelial function. Clin Exp Hypertens 2007; 29: 311-325.

13) Smink PA, Lambers HJ, Gansevoort RT, de Jong PE, Hillege HL, Bakker SJ, de Zeeuw D. Albuminuria, estimated GFR,traditional risk factors and incident cardiovascular disease:the PREVEND (Prevention of Renal and Vascular End stage Disease) Study. Am J Kidney Dis 2012; 60: 804-811.

14) Masson S, Latini R, Anand LS, Barlera S, Angelici L, Vago T, Tognoni G, Cohn JN. Prognostic value of changes in N-terminal pro-brain natriuretic peptide in Val-HeFT (Valsartan Heart Failure Trial). J Am Coll Cardiol 2008; 52: 997-1003.

15) Lipinski MJ, Cauthen CA, Biondi-Zoccai GG, Abbate A, Vrtovec B, Khan BV, Vetrovec GW. Meta-analysis of randomized controlled trials of statins versus placebo in patients with heart failure. Am J Cardiol 2009; 104: 1708-1716.

16) Baigent C, Landray MJ, Reith C, Emberson J, Wheeler DC, Tomson C, Wanner C, Krane V, Cass A, Craig J, Neal B, Jiang L, Hooi LS, Levin A, Agodoa L, Gaziano M, Kasiske B, Walker R, Massy ZA, Feldt-Rasmussen B, Krairittichai U, Ophascharoensuk V, Fellström B, Holdaas H, Tesar V, Wiecek A, Grobbee D, de Zeeuw D, Grönhagen-Riska C, Dasgupta T, Lewis D, Herrington W, Mafham M, Majoni W, Wallendszus K, Grimm R, Pedersen T, Tobert J, Armitage J, Baxter A, Bray C, Chen Y, Chen Z, Hill M, Knott C, Parish S, Simpson D, Sleight P, Young A, Collins R. The effectsof lowering LDL cholesterol with simvastatin plus ezetimibe inpatients with chronic kidney disease, study of heart and renal protection: a randomized placebo-controlled trial. Lancet 2011; 377: 2181-2192.

17) SHARP collaborative group: Study of heart and renal protection (SHARP). Randomized trial to assess the effects of lowering low-density lipoprotein cholesterol among 9,438 patients with chronic kidney disease. Am Heart J 2010; 160: 785-794.

18) Liu M, Wang F, Wang Y, Jin R. Atorvastatin improves endothelial function and cardiac performance in patients with dilated cardiomyopathy: the role of inflamm ation. Cardiovasc Drugs Ther 2009; 23: 369-376.

19) Tousoulis D, Charakida M, Stefanadi E, Siasos G, Latsios G, Stefanadis C. Statins in heart failure. Beyond the lipid lowering effect. Int J Cardiol 2007; 115: 144-1450.

20) Bu DX, Tarrio M, Grabie N, Zhang Y, Yamazaki H, Stavrakis G, Maganto-Garcia E, Pepper-Cunningham Z, Jarolim P, Aikawa M, García-Cardeña G, Lichtman AH. Statin-induced Kruppel-like factor 2 expression in human and mouse T cells reduces inflammatory and pathogenic responses. J Clin Invest 2010; 120: 1961-1970.

21) Tousoulis D, Andreou I, Tsiatas M, Miliou A, Tentolouris C, Siasos G, Papageorgiou N, Papadimitriou CA, Dimopoulos MA, Stefanadis C. Effects of rosuvastatin and allopurinol on circulating endothelial progenitor cells in patients with congestive heart failure: the impact of inflammatory process and oxidative stress. Atherosclerosis 2011; 214: 151-157.

22) Czewski MM, Czewska JM, Duda M. Hypercholesterolaemia exacerbates ventricularremodelling after myocardial infarction in the ratrole of angiotensin II type 1 receptors. Br J Pharmacol 2008; 154: 1640-1648.

23) Blanco S, Vaquero M, Gómez-Guerrero C, López D, Egido J, Romero R. Potential role of angiotensin-converting enzyme inhibitors and statins on early podocyte damage in a model of type 2 diabetes mellitus, obesity, and mild hypertension. Am J Hypertens 2005; 18(4 Pt 1): 557-565.