

Effects of captopril and valsartan on ventricular remodeling and inflammatory cytokines after interventional therapy for AMI

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Abstract. The effects of captopril and valsartan on ventricular remodeling and inflammatory cytokines after interventional therapy for acute myocardial infarction (AMI) were investigated. A total of 94 patients with AMI admitted to Honggang Hospital of Dongying from July 2016 to June 2017 were selected as study subjects. The patients were treated with interventional therapy and randomly divided into the observation group (n=47) and the control group (n=47). The control group received aspirin after operation, while the observation group received captopril and valsartan after operation. Three-dimensional ultrasonography was performed to evaluate ventricular remodeling. The related parameters included left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), left ventricular ejection fraction (LVEF), end-systolic sphericity index/end-diastolic sphericity index (ESSI/EDSI), systolic dyssynchrony index (SDI), diastolic dyssynchrony index (DDI), dispersion end systole (DISPES), DDI-late and DISPED-late. The levels of inflammatory cytokines were determined by enzyme-linked immunosorbent assay (ELISA). The incidence of adverse reactions after treatment was compared. After treatment, LVEF in the control group was significantly lower than that in the observation group, while LVEDV, LVESV and the ratio of early diastolic (E) and late diastolic (A) (E/A) in the control group were significantly higher than those in the observation group ($p < 0.05$). EDSI, DDI-late and DISPED-late in the control group were significantly higher than those in the observation group ($p < 0.05$). ESSI, SDI and DISPES in the control group were significantly higher than those in the observation group ($p < 0.05$). The levels of interleukin-6 (IL-6), high-sensitivity C-reactive protein (hs-CRP) and tumor necrosis factor- α (TNF- α) in the observation group

were significantly lower than those in the control group at 1, 4 and 8 weeks after treatment ($p < 0.05$). The administration of captopril and valsartan after interventional therapy for AMI can effectively improve the cardiac function of patients, improve the synchronism of left ventricular diastole and contraction, and reduce the level of inflammation. It is safe and reliable, and has important clinical significance.

Introduction

Acute myocardial infarction (AMI) is a clinically common cardiovascular disease. Acute myocardial necrosis is caused by persistent ischemia and hypoxia of coronary artery (1). The clinical manifestations of AMI are acute circulatory dysfunction, severe chest pain, increased white blood cell count, ventricular fibrillation, fever, and heart failure. It can usually be divided into transmural and subendocardial myocardial infarction (2). With the continuous improvement of living standards in China, dietary habits are also changing due to the pressure of a fast-paced life and work environment. Thus, the incidence of AMI is high and exhibits a trend towards a younger age group (3). Percutaneous coronary intervention (PCI) is a preferred method for the treatment of AMI, with the advantages of relative safety, and simplicity. However, ventricular remodeling and inflammation problems after PCI operation often trouble patients (4). Ventricular remodeling refers to the process of changes in left ventricular morphology and tissue structure after AMI, which affects the prognosis of patients after PCI. Inflammation problems after PCI may lead to thrombosis and vascular stenosis, increasing the incidence of cardiovascular events, so it should be highly valued (5). In this study, the ventricular remodeling and inflammatory cytokines in patients with AMI were analyzed by different drug intervention after PCI, in order to provide a reference for the postoperative rehabilitation program.

Patients and methods

General data. A total of 94 patients with AMI admitted to Honggang Hospital of Dongying (Dongying, China) from July 2016 to June 2017 were selected. Inclusion criteria were: i) patients who met the AMI diagnostic criteria (6); ii) all patients who underwent PCI surgery and all operations were successful; and iii) patients who signed the informed

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Table I. Comparison of general data between the two groups of patients.

Items	Control group (n=47)	Observation group (n=47)	t/ χ^2	P-value
Sex (male/female)	25/22	23/24	0.043	0.837
Age (years)	40-80	40-75		
Average age (years)	56.36±8.42	56.78±7.57	0.254	0.800
Body mass index (BMI) (Kg/m ²)	23.23±1.05	23.56±1.18	1.432	0.155
Onset time (h)	42.83±4.63	43.14±4.28	0.337	0.737
Previous history (n, %)				
Hypertension	16 (34.04)	18 (38.30)	0.046	0.830
Hyperlipemia	11 (23.40)	13 (27.66)	0.056	0.813
Diabetes	9 (19.49)	7 (14.89)	0.075	0.784

consent. Exclusion criteria were: i) patients accompanied by mental illness; ii) AMI patients who were not treated with PCI; and iii) patients with malignant tumors. The patients were randomly divided into the control group (n=47) and the observation group (n=47). There were no statistically significant differences in general data between the two groups of patients ($p>0.05$), and the data were comparable (Table I). This study was approved by the Ethics Committee of Honggang Hospital of Dongying. Signed informed consents were obtained from the patients or guardians.

Methods

Treatment. The patients received PCI, and the patients in the control group were given oral aspirin enteric-coated tablets (Yangtze River Pharm Co., Ltd., Taizhou, China) at a dose of 100 mg, once a day, for 1 year. On the basis of the treatment of the control group, the observation group was given 18 mg captopril (Sino-American Shanghai Squibb Pharmaceuticals Co., Ltd.; approval no. NMPN H31022986) per day and 80 mg valsartan (Hunan Qianjin Xiangjiang Pharmaceutical Industry Co., Ltd. (Hunan, China) approval no. NMPN H20103521) per day for 6 consecutive months.

Real-time three-dimensional ultrasonography. After 24 weeks of treatment, ACUSON SC2000 ultrasonic diagnostic apparatus (Siemens AG, Munich, Germany) was used to examine the two groups of patients. The 4Z1c volumetric probe (frequency 2.8 MHz) was used. The scanning angle was 90°x90°; the scanning depth was 15-16 cm, and the volume rate was ≥ 12 frames/sec. The subjects were instructed to take the left-lateral position, and the synchronous lead electrocardiogram was connected to record their electrocardiogram. At the end of expiration, dynamic three-dimensional images of three complete cardiac cycles were collected and stored. Then the related parameters were recorded, including left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), left ventricular ejection fraction (LVEF), end-systolic sphericity index/end-diastolic sphericity index (ESSI/EDSI), systolic dyssynchrony index (SDI), diastolic dyssynchrony index (DDI), dispersion end systole (DISPES), DDI-late and DISPED-late.

Concentration detection of interleukin-6 (IL-6), high-sensitivity C-reactive protein (hs-CRP) and tumor necrosis factor- α (TNF- α). The concentration levels of IL-6, hs-CRP and TNF- α were determined by enzyme-linked immunosorbent assay (ELISA) at 1, 4 and 8 weeks after operation. All relevant kits were provided by Shanghai HaoBen Biotechnology Co., Ltd. In strict accordance with the instructions of the kits, the optical density (OD) value was read at a wavelength of 450 nm with a microplate reader (Elx800; BioTek Instruments, Inc., Winooski, VT, USA), and the concentration levels of IL-6, hs-CRP and TNF- α were calculated.

Evaluation criteria. At 24 weeks after treatment, real-time three-dimensional ultrasonography was performed for two groups of patients. The LVEF, LVEDV, LVESV, EDSI, DDI-late, DISPED-late, ESSI, SDI and DISPES were recorded.

The serum levels of IL-6, hs-CRP and TNF- α in two groups of patients were detected by ELISA at 1, 4 and 8 weeks after operation. The incidence of adverse reactions was compared between the two groups of patients, including severe bleeding, thrombocytopenia, abnormal liver function and gastrointestinal reaction.

Statistical analysis. The data were processed by Statistical Product and Service Solutions (SPSS) 19.0 (SPSS Inc., Chicago, IL, USA) software. The measurement data were expressed as mean \pm standard deviation ($\bar{x}\pm s$), and detected by t-test. The enumeration data were expressed as rate, and detected by χ^2 test. $P<0.05$ suggested that the difference was statistically significant.

Results

Comparison of cardiac functions between the two groups of patients. After 24 weeks, LVEF in the control group was significantly lower than that in the observation group, while LVEDV, LVESV and the ratio of early diastolic (E) and late diastolic (A) (E/A) in the control group were significantly higher than those in the observation group ($p<0.05$) (Table II).

Table II. Comparison of cardiac functions between two groups of patients after treatment ($\bar{x}\pm s$).

Groups	n	LVEF (%)	LVEDV (ml)	LVESV (ml)	E/A
Observation group	47	61.73±3.68	90.54±3.64	36.76±3.08	1.48±0.28
Control group	47	55.78±3.56	97.26±3.86	42.87±3.15	2.62±0.35
t-test		7.967	8.683	9.508	17.437
P-value		<0.001	<0.001	<0.001	<0.001

LVEF, left ventricular ejection fraction; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; E/A, ratio of early diastolic (E) and late diastolic (A).

Table III. Comparison of left ventricular diastolic functions with good quality in the two groups of patients (% , $\bar{x}\pm s$).

Groups	n	EDSI	DDI-late	DISPED-late
Observation group	47	41.56±3.19	5.19±0.78	21.45±1.14
Control group	47	46.64±3.73	7.63±0.95	25.68±1.53
t-test		7.096	13.609	15.199
P-value		<0.001	<0.001	<0.001

EDSI, end-diastolic sphericity index; DDI, diastolic dyssynchrony index; DISPES, dispersion end systole.

Table IV. Comparison of left ventricular systolic functions after treatment in the two groups of patients (% , $\bar{x}\pm s$).

Groups	n	ESSI	SDI	DISPES
Observation group	47	36.54±3.08	5.36±1.25	19.78±3.09
Control group	47	41.38±3.27	7.89±1.62	26.82±3.53
t-test		7.387	8.477	10.288
P-value		<0.001	<0.001	<0.001

ESSI, end-systolic sphericity index; SDI, systolic dyssynchrony index; DISPES, dispersion end systole.

Table V. Comparison of TNF- α levels between the two groups of patients after treatment (pg/ml, $\bar{x}\pm s$).

Groups	n	At 1 week after operation	At 4 weeks after operation	At 8 weeks after operation
Observation group	47	59.67±3.23	32.35±3.28	23.68±3.24
Control group	47	67.27±3.64	49.47±3.36	32.83±3.78
t-test		10.707	24.996	12.600
P-value		<0.001	<0.001	<0.001

TNF- α , tumor necrosis factor- α .

Comparison of left ventricular diastolic functions between the two groups of patients. After 24 weeks, EDSI, DDI-late and

Table VI. Comparison of hs-CRP levels between the two groups of patients after treatment (mg/l, $\bar{x}\pm s$).

Groups	n	At 1 week after operation	At 4 weeks after operation	At 8 weeks after operation
Observation group	47	6.05±1.13	3.02±0.61	1.63±0.344
Control group	47	10.72±2.18	3.64±0.78	2.52±0.47
t-test		13.039	4.293	10.518
P-value		<0.001	<0.001	<0.001

hs-CRP, high-sensitivity C-reactive protein.

Table VII. Comparison of IL-6 levels between the two groups of patients after treatment (ng/l, $\bar{x}\pm s$).

Groups	n	At 1 week after operation	At 4 weeks after operation	At 8 weeks after operation
Observation group	47	12.89±2.23	7.52±1.15	4.83±1.04
Control group	47	16.36±2.28	9.74±1.47	7.79±1.15
t-test		7.459	8.155	13.088
P-value		<0.001	<0.001	<0.001

IL-6, interleukin-6.

DISPED-late in the control group were significantly higher than those in the observation group ($p<0.05$) (Table III).

Comparison of left ventricular systolic functions between the two groups of patients. After 24 weeks, ESSI, SDI and DISPES in the control group were significantly higher than those in the observation group ($p<0.05$) (Table IV).

Comparison of TNF- α levels between the two groups of patients. TNF- α levels in the observation group were significantly lower than those in the control group at 1, 4 and 8 weeks after treatment ($p<0.05$) (Table V).

Comparison of hs-CRP levels between the two groups of patients. hs-CRP levels in the observation groups were

Table VIII. Comparison of the incidence of adverse reactions between the two groups of patients (n, %).

Groups	n	Severe bleeding	Thrombocytopenia	Abnormal liver function	Gastrointestinal reaction
Observation group	47	1 (2.13)	2 (4.26)	1 (2.13)	1 (2.13)
Control group	47	3 (6.38)	4 (8.51)	0 (0.00)	2 (4.26)
χ^2		0.261	0.178	0.001	0.001
P-value		0.609	0.673	0.999	0.999

significantly lower than those in the control group at 1, 4 and 8 weeks after treatment ($p < 0.05$) (Table VI).

Comparison of IL-6 levels between the two groups of patients. IL-6 levels in the observation group were significantly lower than those in the control group at 1, 4 and 8 weeks after treatment ($p < 0.05$) (Table VII).

Comparison of the incidence of adverse reactions between the two groups of patients. There was no significant difference in the incidence of adverse reactions between the two groups of patients ($p > 0.05$) (Table VIII).

Discussion

AMI is a clinically common cardiovascular disease. The rupture of coronary atherosclerotic plaques usually causes thrombosis, which blocks coronary artery vessels and causes myocardial infarction, leading to arrhythmia, atrial fibrillation, hypotension, post-sternal pain and other symptoms in patients (7). Usually, when the area of myocardial infarction exceeds 20%, hemodynamics are obviously decompensated, and when the infarction area exceeds 40%, cardiogenic shock occurs (8). There are many causes of AMI, including emotion, overwork, cold stimulation, overeating, smoking, heavy drinking, and constipation (9). Clinically, PCI treatment can recanalize the coronary artery, so as to rapidly recover the blood perfusion of coronary artery of the patients, and rebuild the blood supply (10).

Ventricular remodeling in patients after PCI mainly involves changes in cardiomyocytes and extracellular matrix, such as the increased number and size as well as apoptosis of cardiomyocytes. Ventricular remodeling includes myocardial reactive hypertrophy, left ventricular deformation, myocardial thinning of ventricular wall, diminished ventricular compliance, decreased cardiac function, and intraventricular systolic and diastolic asynchrony (11,12). The results of this study showed that, after treatment, LVEF in the control group was significantly lower than that in the observation group, while LVEDV, LVESV and E/A in the control group were significantly higher than those in the observation group. EDSI, DDI-late and DISPED-late in the control group were significantly higher than those in the observation group. ESSI, SDI and DISPES in the control group were significantly higher than those in the observation group ($p < 0.05$). This is because captopril is an angiotensin-converting enzyme inhibitor that reduces ventricular filling pressure and vascular resistance. Captopril treatment after PCI can reduce left ventricular load and early myocardial

remodeling, protect cardiac ejection fraction, and improve myocardial compliance and relaxation ability (13). Valsartan is an angiotensin II receptor antagonist, with the effect of dilating blood vessels to lower blood pressure, which can increase coronary perfusion in infarcted and non-infarcted areas, thereby reducing left ventricular end-diastolic pressure and reducing LVEDV and LVESV (14). The combination of the two drugs can effectively reduce the cardiac load and relieve the condition of limited ventricular filling, which significantly inhibits the enlargement of left ventricular end-diastolic diameter after PCI in patients with AMI, reduces the expansion of infarct size, and has reversal effects on the proliferation of cardiomyocytes and interstitial collagen deposition, thereby improving the myocardial and vascular remodeling, and reducing left atrial pressure and E/A value (15). Real-time three-dimensional ultrasonography revealed decreased DDI in the observation group showing that the recovery of diastolic function in the observation group was superior to that in the control group, and SDI in the observation group was significantly lower than that in the control group, which meant that the degree of systolic dysfunction was significantly relieved, and there was synchronism of ventricular diastole and contraction.

During catheterization, balloon dilation and other operations, PCI inevitably causes damage to the blood vessels of the patients, which results in the exfoliation of vascular endothelium, and releases a large number of inflammatory cytokines (16). hs-CRP is a commonly used marker of inflammatory response, which can directly reflect the inflammatory status of patients with AMI, and can be used as a predictor of prognostic cardiovascular events in patients with AMI (17). TNF- α is the earliest inflammatory mediator produced by the body, which may initiate and trigger inflammatory response, and cause a cascade reaction. When there are myocardial ischemia and hypoxia, cardiomyocytes and interstitial cells secrete a large amount of TNF- α , induce apoptosis of cardiomyocytes, and activate inflammatory response (18). As an acute phase response lymphocyte factor, IL-6 can play various roles. The increased secretion of IL-6 after PCI induces inflammatory cell adhesion and aggregation to promote inflammation, but at the same time, it plays the role of inflammatory resistance (19). The results of this study showed that the levels of IL-6, hs-CRP and TNF- α in the observation group were significantly lower than those in the control group at 1, 4 and 8 weeks after treatment ($p < 0.05$). This is because aspirin is a cyclooxygenase inhibitor, which blocks the platelet aggregation by inhibiting the enzyme activity of acetylated cyclooxygenase-1 (COX-1), thereby reducing the secretion of inflammatory cytokines (IL-6, hs-CRP and TNF- α). Captopril

combined with valsartan may effectively protect the vascular endothelial function, and inhibit the overactivation of nuclear factor κ B and Th1 cells in endothelial cells, thereby reducing the expression of IL-6, hs-CRP and TNF- α . As the time of administration was increased, the inflammation in patients was effectively suppressed. The levels of IL-6, hs-CRP and TNF- α were significantly decreased, and the degree of inflammation in patients with AMI was reduced (20).

In summary, captopril and valsartan administration in patients with AMI after PCI may effectively prevent the left ventricular remodeling, improve left ventricular function, and reduce the degree of inflammation. It is worthy of clinical promotion and application.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XG wrote the initial draft of the manuscript, revised and finalized this study. XG and RZ designed the study and performed the research. QL collected and analyzed the data of this study. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Honggang Hospital of Dongying (Dongying, China). Signed informed consents were obtained from the patients or guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Woods KL, Ketley D, Agusti A, Hagn C, Kala R, Karatzas NB, Leizorowicz A, Reikvam A, Schilling J, Seabra-Gomes R, *et al*: Use of coronary angiography and revascularization procedures following acute myocardial infarction. A European perspective. *Eur Heart J* 19: 1348-1354, 1998.
- Bolca O, Güngör B, Özcan KS, Karadeniz FO, Sungur A, Koroğlu B, Bakhshyaliev N, Yelgeç NS, Karataş B, İpek G, *et al*: The neutrophil-to-lymphocyte ratio is associated with bare-metal stent restenosis in STEMI patients treated with primary PCI. *Coron Artery Dis* 26: 402-408, 2015.
- Schlienger RG, Jick H and Meier CR: Use of nonsteroidal anti-inflammatory drugs and the risk of first-time acute myocardial infarction. *Br J Clin Pharmacol* 54: 327-332, 2002.
- Cung TT, Morel O, Cayla G, Rioufol G, Garcia-Dorado D, Angoulvant D, Bonnefoy-Cudraz E, Guérin P, Elbaz M, Delarche N, *et al*: Cyclosporine before PCI in patients with acute myocardial infarction. *N Engl J Med* 373: 1021-1031, 2015.
- Reichlin T, Cullen L, Parsonage WA, Greenslade J, Twerenbold R, Moehring B, Wildi K, Mueller S, Zellweger C, Mosimann T, *et al*: Two-hour algorithm for triage toward rule-out and rule-in of acute myocardial infarction using high-sensitivity cardiac troponin T. *Am J Med* 128: 369-79.e4, 2015.
- Afana M, Brinjikji W, Cloft H and Salka S: Hospitalization costs for acute myocardial infarction patients treated with percutaneous coronary intervention in the United States are substantially higher than Medicare payments. *Clin Cardiol* 38: 13-19, 2015.
- Reichlin T, Twerenbold R, Wildi K, Rubini Gimenez M, Bergsma N, Haaf P, Druey S, Puelacher C, Moehring B, Freese M, *et al*: Prospective validation of a 1-hour algorithm to rule-out and rule-in acute myocardial infarction using a high-sensitivity cardiac troponin T assay. *CMAJ* 187: E243-E252, 2015.
- Risenfors M, Herlitz J, Berg CH, Dellborg M, Gustavsson G, Gottfridsson C, Lomsby M, Swedberg K and Hjalmarsson A: Early treatment with thrombolysis and beta-blockade in suspected acute myocardial infarction: Results from the TEAHAT Study. *J Intern Med Suppl* 734: 35-42, 1991.
- Dauerman HL, Pinto DS, Ho KK, Gibson CM, Kuntz RE, Cohen DJ, Baim DS and Carrozza JP Jr: Outcome of patients with acute myocardial infarction who are ineligible for primary angioplasty trials. *Catheter Cardiovasc Interv* 49: 237-243, 2000.
- Akin I and Nienaber CA: Treatment of coronary in-stent restenosis-evidence for universal recommendation? *J Thorac Dis* 7: 1672-1675, 2015.
- Shimonaga T, Kurisu S, Watanabe N, Ikenaga H, Higaki T, Iwasaki T, Ishibashi K, Dohi Y, Fukuda Y and Kihara Y: Myocardial injury after percutaneous coronary intervention for in-stent restenosis versus de novo stenosis. *Intern Med* 54: 2299-2305, 2015.
- Westman PC, Lipinski MJ, Luger D, Waksman R, Bonow RO, Wu E and Epstein SE: Inflammation as a driver of adverse left ventricular remodeling after acute myocardial infarction. *J Am Coll Cardiol* 67: 2050-2060, 2016.
- Antonelli L, Katz M, Bacal F, Makdisse MRP, Correa AG, Pereira C, Franken M, Fava AN, Serrano Junior CV and Pesaro AEP: Heart failure with preserved left ventricular ejection fraction in patients with acute myocardial infarction. *Arq Bras Cardiol* 105: 145-150, 2015.
- Nikolaou NI, Arntz HR, Bellou A, Beygui F, Bossaert LL, Cariou A and Danchin N: Initial management of acute coronary syndromes section Collaborator: European Resuscitation Council Guidelines for Resuscitation 2015 Section 8. Initial management of acute coronary syndromes. *Resuscitation* 95: 264-277, 2015.
- Wang L, Liu G, Liu J, Zheng M and Li L: Effects of no-reflow phenomenon on ventricular systolic synchrony in patients with acute anterior myocardial infarction after percutaneous coronary intervention. *Ther Clin Risk Manag* 12: 1017-1022, 2016.
- Güngör B, Karataş MB, İpek G, Özcan KS, Çanga Y, Onuk T, Keskin M, Hayıroğlu Mİ, Karadeniz FO, Sungur A, *et al*: Association of contrast-induced nephropathy with bare metal stent restenosis in STEMI patients treated with primary PCI. *Ren Fail* 38: 1167-1173, 2016.
- Li H, Jiang Z, Liu X and Yang Z: Higher plasma level of STIM1, OPG are correlated with stent restenosis after PCI. *Int J Clin Exp Med* 8: 21089-21097, 2015.
- Yamagata K, Xie Y, Suzuki S and Tagami M: Epigallocatechin-3-gallate inhibits VCAM-1 expression and apoptosis induction associated with LC3 expressions in TNF α -stimulated human endothelial cells. *Phytomedicine* 22: 431-437, 2015.
- Hozumi H, Russell J, Vital S and Granger DN: IL-6 mediates the intestinal microvascular thrombosis associated with experimental colitis. *Inflamm Bowel Dis* 22: 560-568, 2016.
- Sepehri Z, Masoumi M, Ebrahimi N, Kiani Z, Nasiri AA, Kohan F, Sheikh Fathollahi M, Kazemi Arababadi M and Asadikaram G: Atorvastatin, losartan and captopril lead to upregulation of TGF- β , and downregulation of IL-6 in coronary artery disease and hypertension. *PLoS One* 11: e0168312, 2016.



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