Analysis on mechanism of ATP-sensitive K^+ channel opener natakalim improving congestive heart failure after myocardial infarction

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Abstract. The action mechanism of natakalim, a novel ATP-sensitive potassium channel opener, was studied in ameliorating the congestive heart failure (CHF) after myocardial infarction. A total of 25 healthy Wistar male rats (age, 10 weeks; average weight, 300 g) were selected, and the CHF models after acute myocardial infarction (AMI) were prepared by ligation of left anterior descending branch. They were randomly divided into the sham operation group, the model group and the groups of 1, 3 and 9 mg/kg/day natakalims. Each group had 5 mice that were sacrificed after 8 weeks. We compared left ventricular end-diastolic diameter (LVEDD), left ventricular ejection fraction (LVEF), N-terminal prohormone of brain natriuretic peptide (NT-proBNP), left ventricular mass index, myocardial cell cross-sectional area, myocardial collagen content, plasma endothelin-1 (ET-1) and endothelial nitric oxide synthase (eNOS) levels. Compared with the sham operation, the LVEDD and NT-proBNP in the model group and each natakalim group were elevated. LVEF decreased significantly, while the left ventricular mass index, myocardial cell cross-sectional area, myocardial collagen content, plasma ET-1 and eNOS levels increased. Natakalim intervention improved the above changes and the improvement effect of 3 mg/kg/day group was the highest. The mechanism of natakalim against the endothelin system can be explained by the fact that inhibiting ET-1 synthesis can reduce the ET-1 levels in circulation leading to the release of NO and PGI2. Inhibition of the vasoconstriction effect of ET-1 can improve the hemodynamics of high-load status and ameliorate the cardiac systolic and diastolic functions. In conclusion, natakalim can improve the ventricular remodeling of CHF after AMI, and 3 mg/kg/day was the most effective dose.

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Introduction

Congestive heart failure (CHF) is a condition in which the heart does not pump as well as it should. CHF can be considered the final stage of a variety of organic diseases of the heart, with higher mortality. The occurrence rate of CHF is higher after acute myocardial infarction (AMI) and the prognosis is poorer (1). The main mechanism of AMI-induced CHF is the ventricular remodeling, in which the vascular endothelial cells may play an important role (2). Previous findings revealed that the endothelial cells as the body's largest endocrine organ with abnormally active functions could, not only sense the hemodynamic changes and blood transfer signal, but also synthesize and secrete a variety of vasoactive substances (3). These vasoactive substances included NO, cell adhesion molecule-1 (PGI2), endothelin-1 (ET-1), angiotensin II (Ang II), platelet activating factor, plasminogen activator (PA) [such as tissue type PA (tPA)] and intercellular adhesion molecule-1 (ICAM-1) (4-7). They fulfil several functions in human body: i) Maintaining the dynamic equilibrium between vascular relaxing factor and shrinkage factor (8); ii) maintaining the dynamic equilibrium between the coagulation and fibrinolytic system (9); iii) inhibiting the proliferation of vascular smooth muscle cells; iv) involving in the inflammation and immune response (10); and v) regulating the lipid oxidation and vascular permeability (11).

ATP-sensitive potassium (K^+) channel (K_{ATP}) is the link between the cell electrophysiology and the metabolism. There are a few scholars who have proposed (12) that the selective activation of endothelial cell SUR2B/Kir6.1 K_{ATP} could correct the endothelial dysfunction and restore endothelial normal function, which was taken as an important new target for treatment of cardiovascular disease. Based on this idea, in this study, the action mechanism of natakalim, a novel K_{ATP} channel opener, was analyzed in ameliorating the post-infarction CHF.

Materials and methods

Animals. Twenty-five healthy Wistar male rats (age, 10 weeks; average weight, 300 g) were selected under regular feeding. The rats were randomly selected and divided into the sham

Table I. Comparison of LVEDD, LVEF and NT-proBNP.

Groups	LVEDD (mm)		LVEF (%)		NT-proBNP (pg/ml)	
	Before experiment	After experiment	Before experiment	After experiment	Before experiment	After experiment
Sham operation	23.4±2.8	23.6±2.5	62.4±3.5	62.3±3.3	52.6±10.3	53.3±11.1
Model	23.5±2.7	35.5±2.9	62.5±3.6	42.7±3.2	55.3±11.2	432.7±34.5
1 mg/kg/day	23.2±2.5	34.3±2.7	62.3±3.7	45.5±3.2	50.7±11.9	405.3±36.6
3 mg/kg/day	22.8±2.6	33.6±2.8	62.4±3.6	47.6±3.4	52.4±12.2	362.2±38.2
9 mg/kg/day	22.9±2.8	34.0±2.9	62.6±3.8	45.9±3.4	51.9±10.6	396.4±39.7
F-value	0.462	7.529	0.629	8.636	0.935	23.524
P-value	0.328	0.008	0.744	<0.001	0.766	<0.001

LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal prohormone of brain natriuretic peptide.

operation group, the model group and the groups of 1, 3 and 9 mg/kg/day natakalims (n=5). The gastric infusion was given to rats in the model group and each of the natakalim groups from the third day and after. Natakalim was dissolved in the normal saline, with the perfusion volume of 0.5 ml/100 g, the model group received an equal volume of distilled water for 8 weeks continuously, once a day.

The study was approved by the Animal Ethics Committee of Xiangyang No. 1 People's Hospital.

CHF models after AMI. AMI were conducted by ligation of left anterior descending coronary artery. The main steps were the following: i) Rats were under anesthesia by intraperitoneal injection of pentobarbitalum natricum (40 mg/kg); ii) they were in supine position and fixed on the operating table; iii) they underwent tracheostomy and were connected to small animal respirators, with the respiratory rate of 60/min and the inspiratory to expiratory ratio of 1:1.5; and iv) electrocardiogram (ECG) electrode needle was buried under subcutaneous area in limbs and normal ECG was recorded. The hair in the operation area was cleared, disinfected by iodophor and the skin was cut along the left sternum. Sternum was cut between third and fourth ribs on the left and the intercostal muscle was bluntly dissected layer by layer to expose the heart. Hemostat was used to carefully distract the intercostal incision and the ophthalmic forceps was used to strip the pericardium. Cotton swab was used to gently pick up the left atrial appendage. The left anterior descending coronary artery was passed through by 7-0 atraumatic needle at the site, 2 mm from the left anterior descending coronary artery starting site between the pulmonary cone and left aurcle. Then it was under ligation together with a small bundle of myocardia. ECG showed that lead II was elevated at ST segment, while it was clearly seen that the ligation region became white and pulse was weakened, proving that the ligation was successful. In the sham operation group, the left anterior descending coronary artery site was only passed through by the needle between the left aurcle left edge and pulmonary arterial cone without ligation, while the rest of the procedure was the same as the model group. A thin plastic hose was placed in before closing thoracic cavity to absorb gas after the closure and to drain the oozy blood in order to recover intrathoracic negative pressure. Subsequently, the thoracic cavity was closed layer by layer. After the spontaneous breathing was established in rats, the thoracic cavity was unplugged and the trachea and neck skin were sutured. After operation, 200,000 μ/day penicillin was administered for three days to prevent infection.

Observation indicators and test methods. After 8 weeks, rats were sacrificed and left ventricular end-diastolic diameter (LVEDD), left ventricular ejection fraction (LVEF), N-terminal prohormone of brain natriuretic peptide (NT-proBNP), left ventricular mass index, myocardial cell cross-sectional area, myocardial collagen content, plasma ET-1 and endothelial nitric oxide synthase (eNOS) levels were compared among groups. LVEDD and LVEF were measured by medium-sized animal dedicated cardiac ultrasound instrument (Beijing Liuyi Instrument Factory, Beijing, China). Tail vein blood (5 ml) was collected and centrifuged at 3,000 rpm for 20 min and stored at -80˚C. NT-proBNP and eNOS were tested by ELISA kits (Sigma-Aldrich, St. Louis, MO, USA) and ET-1 was tested by radioimmunoassay kits (Beijing Institute of East Asian RIA, Beijing, China). Rat hearts were taken out and quickly placed in ice-cold saline at -4˚C and then rinsed and dried by filter paper. An electronic scale was used to accurately weigh the hearts, the left and right ventricles were divided along the ventricle and their weights were measured separately. The ratio of the left ventricular weight to body weight was calculated. The paraffin tissue sections were prepared and stained by hematoxylin and eosin. Medical image analysis system was used to measure the individual cell cross-sectional area and the mean values were recorded. Masson's staining was performed and the myocardial collagen fibers were stained in green, while the myocardial cells were stained in red. Image-Pro Plus 6.0 image analysis software was used to measure the myocardial interstitial collagen volume fraction.

Statistical analysis. SPSS 19.0 statistical software (Chicago, IL, USA) was used for statistical analysis. Quantitative data were expressed as mean ± standard deviation and the comparison between the two groups was conducted using one-way ANOVA. Qualitative data were expressed as number of cases or percentage (%) and the comparison between the two groups was done using χ^2 test method. P<0.05 was considered to indicate a statistically significant difference.

Results

Comparison of LVEDD, LVEF and NT-proBNP. Before the experiment, when LVEDD, LVEF and NT-proBNP were compared among the groups, the differences were not statistically significant (P>0.05). After the experiment LVEDD and NT-proBNP values in the model group were the highest compared to other groups followed by the 1, 9 and 3 mg/kg/day groups. LVEF decreased in all groups, but the lowest value was recorded for the model group, followed by the 1 and 9 mg/kg/day groups. The highest value for LVEF was in the 3 mg/kg/day group. Differences were statistically significant (P<0.05) (Table I).

Table II. Comparison of left ventricular mass index, myocardial cell cross-sectional area and collagen content (%).

Groups	Left ventricular mass index	Cell cross-sectional area	Collagen
Model	8.5±1.3	178.6±23.4	6.4±1.5
1 mg/kg/day	8.2±1.4	152.3±21.2	6.0±1.6
3 mg/kg/day	6.9±1.2	120.5±20.7	4.7±1.3
9 mg/kg/day	8.0±1.5	146.6±22.3	5.5±1.4
F-value	6.458	6.935	6.625
P-value	0.026	0.021	0.023

Comparison of left ventricular mass index, myocardial cell cross-sectional area and collagen content. Compared with the sham operation group, the left ventricular mass index, myocardial cell cross-sectional area and collagen levels were higher in all other groups. The highest value was recorded in the model group, followed by the 1 and 9 mg/kg/day groups. The lowest values were observed in the 3 mg/kg/day group.

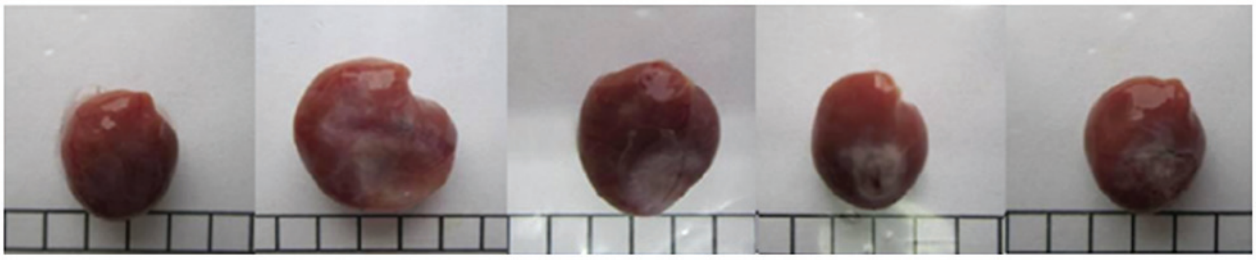

Figure 1. Comparison of left ventricular mass indexes. From left to right: Sham operation, model, and combined 1 and 9 mg/kg/day groups.

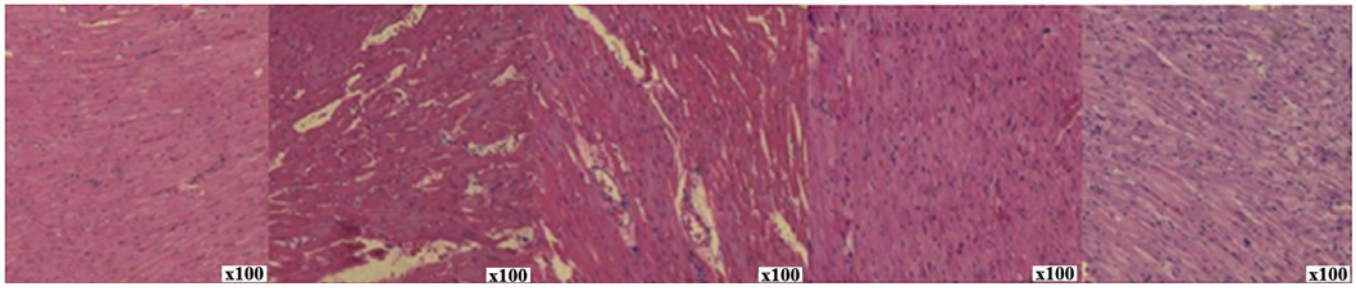

Figure 2. H&E staining of myocardial cell cross-section area (magnification, x100). From left to right: Sham operation, model, and 1, 3 and 9 mg/kg/day groups. H&E, hematoxylin and eosin.

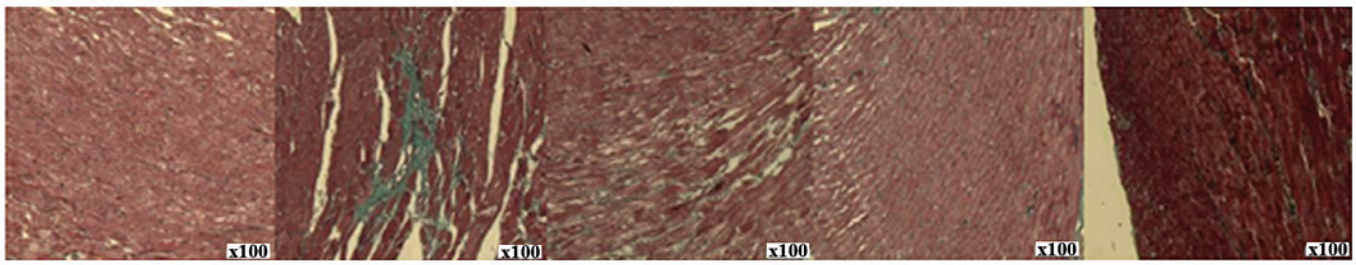

Figure 3. Myocardial collagen Masson's staining (magnification, x100). From left to right: Sham operation, model, and 1, 3 and 9 mg/kg/day groups.

Table III. Comparison of ET-1 and eNOS levels.

Groups	ET-1 (pg/ml)		eNOS (μg/ml)	
	Before experiment	After experiment	Before experiment	After experiment
Sham operation	213.4±42.6	217.5±43.2	6.6±2.2	6.5±2.3
Model	206.3±43.3	356.8±55.6	6.5±2.3	17.2±2.2
1 mg/kg/day	215.5±41.4	321.5±51.8	6.3±2.4	14.3±2.6
3 mg/kg/day	208.7±43.5	277.9±48.2	6.4±2.5	8.8±2.4
9 mg/kg/day	210.2±42.7	303.6±56.4	6.6±2.3	11.5±2.6
F-value	0.526	7.012	0.864	7.714
P-value	0.649	0.019	0.963	0.005

ET-1, endothelin-1; eNOS, endothelial nitric oxide synthase.

All differences were statistically significant (P<0.05) (Table II and Figs. 1-3).

In the model group, the myocardial fiber arrangement was thickened, disordered and even ruptured. Myocardial hypertrophy, myocardial interstitial significant hyperplasia, inflammatory cell infiltration and significant increase in myocardial cell cross-sectional area occurred. Compared with the model group, the cell cross-sectional area in each natakalim group was reduced, and the reduction in the 3 mg/kg/day group was the most obvious.

Comparison of ET-1 and eNOS levels. Prior to the experiment, differences between the ET-1 and eNOS levels in different groups did not reveal any statistically significant differences (P>0.05). In addition to the sham operation, after the experiment ET-1 and eNOS levels were considerably elevated in all the groups. The highest values were recorded in the model group, followed by the 1 and 9 mg/kg/day groups. The lowest values were observed in the 3 mg/kg/day group. Differences were statistically significant (P<0.05) (Table III).

Discussion

Previous findings revealed that natakalim was capable of protecting the aortic endothelial cells with hypoxia and homocysteine injury, reversing the ventricular remodeling in animal models with abdominal aorta coarctation-induced overloading pressure and preventing the development and progression of ventricular remodeling to heart failure (13). The mechanism was related to the correction of endothelial dysfunction and protection of endothelial function. Our results showed that compared with the sham operation group, the LVEDD and NT-proBNP in the model group and each of the natakalim groups were elevated. LVEF decreased, the left ventricular mass index, myocardial cell cross-sectional area, myocardial collagen content, plasma ET-1 and eNOS levels increased. Natakalim positively affected all of the above-mentioned indexes. This effect was more significant in the 3 mg/kg/day group. LVEDD, LVEF, left ventricular mass index and microcosmic myocardial cell cross-sectional area and myocardial collagen content, NT-proBNP reflected the neuroendocrine regulation. ET-1 and eNOS are two important cytokines secreted by endothelial cells and play important roles in ventricular remodeling.

Under myocardial ischemia and hypoxia, a large number of ATP molecules is consumed and K^+ concentration inside cells is significantly reduced. This can open the K_{ATP}, shorten the myocardial action potential duration, reduce Ca^{2+} influx, which is an important self-protection mechanism (14) against myocardial ischemia and hypoxia. Watanuki *et al* showed that after being pre-incubated for 60 min with 5 mg/l pertussis toxin in the isolated guinea pig ventricular myocytes, ET-1-induced K^+ influx was significantly eliminated (15). ET-1 and its receptor influenced the metabolic state of the cells and inhibited the openness of the K_{ATP} by intracellular signal transduction.

The mechanism of natakalim against the endothelin system may be explained by the fact that inhibiting ET-1 synthesis and its release, could reduce the ET-1 levels in the circulation (16) leading to the release of NO and PGI2. Inhibition of the vasoconstriction effect of ET-1, improved the hemodynamics of high-load status (17) and ameliorated the cardiac systolic and diastolic functions (18). It was manifested as the reversal of cardiac remodeling, restoration of reduced heart function and prevention of the development and progression of heart failure in animal models.

NO generated by catalysis of eNOS can relax the blood vessels, reduce the cardiac preload and afterload which lead to myocardial protection (19). It can also directly enhance the dilatation effect of ventricular muscle, maintain cardiac output through the left ventricle Frank-Starling regulation mechanism and be beneficial for the heart pump function under physiological and pathological states (20). Scherrer-Crosbie *et al* found out that deletion of eNOS gene in the mouse model with myocardial infarction, significantly reduced eNOS levels. Compared to wild-type mice, these mice developed a more severe myocardial infarction and ventricular remodeling (21). Natakalim could promote the eNOS protein expression, raise the level of endothelium-derived NO, increase eNOS-NO pathway activity (22), reduce inducible NOS (iNOS) protein expression and reduce the generation of a large number of iNOS-induced NOs and inhibit iNOS-NO pathway (23).

We concluded that natakalim can improve the ventricular remodeling of CHF after AMI, and 3 mg/kg/day was the most

effective dose. This may be related to the inhibition effect of ET-1 and promotion of NO and eNOS activities.

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