**Laboratory Research**

**Effect of captopril on myocardial energy metabolism in chronic pressure overload rats**

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**Objective** To investigate the effects of captopril on cardiac function and levels of energy-rich phosphates in pressure overload induced left ventricular hypertrophy rats. **Methods** One hundred and twenty SD rats were randomly divided into three groups: sham operation group (SH group, n=40), coarctation of abdominal aorta group (CAA group, n=40) and captopril treatment 1mg · 100g⁻¹·d⁻¹ group (CAP group, n=40). Left ventricular end-diastolic pressure (LVEDP), left ventricular mass index (LVMI), levels of energy-rich phosphates and morphological changes of the myocardial mitochondria were compared at the 6th and 8th week after operation. **Results** At 6th week, in CAA group, LVMI and LVEDP were increased and ±dp/dtmax was decreased, while ATP and ADP were decreased and AMP was increased (P<0.01). These changes were much obvious at 8th week (P<0.01). Compared with those of CAA group, the parameters of heart function and energy-rich phosphates (ATP, ADP, AMP, TAN) in CAP group were improved significantly (P<0.01) at the 6th and 8th week. In CAP group, the parameters of heart function and energy-rich phosphates (ADP, AMP, TAN) were much better at 8th week than those at 6th week. The morphological change of mitochondria was less in CAP group than that in CAA group. **Conclusion** Captopril significantly improves myocardial energy metabolism in pressure overload rats and protects the function of myocardial mitochondria (*J Geriatr Cardiol* 2010; 7:176-179).

**Key words** pressure overload; myocardial energy metabolism; energy-rich phosphate

**Introduction**

Angiotensin-converting enzyme inhibitors (ACE-I) are well-known as antihypertensive agents. In addition, they exert several cardioprotective effects such as prevention and regression of left ventricular hypertrophy or reduction of infarct size in both clinical and experimental studies, and represent an important therapeutic approach in the treatment of congestive heart failure. However, the mechanism of the beneficial effect of ACE-I remains to be fully understood.

The energy dysmetabolism of cardiac muscle cause myocardial cell damage and promote the development of heart failure. Most previous studies dealing with the effects of ACE-I on the isolated myocardial heart have focused on functional parameters, but only a few on metabolism, in particular on energy reserves. This study was designed to investigate the effects of captopril on cardiac function and levels of energy-rich phosphates in pressure overload induced left ventricular hypertrophy rats.

**Methods**

**Animal model preparation**

Totally, 120 SD rats were randomly divided into three groups: sham operation group (SH) (n=40), coarctation of abdominal aorta group (CAA) (n=40) and Captopril group (CAP) (n=40). Animal model preparation: For rats in SH group and CAP group, after intraperitoneal injection of 10% chlora hydrate anesthesia (300mg/kg), about 1 cm of the abdominal artery above the top of left renal artery was exposed. Then, a stainless probe of 0.7-0.8 mm in diameter was placed along with the abdominal aorta and were ligated, then, the probe was pull out and the abdominal aortic stenosis was induced (50%~60%). The incision was finally sutured and penicillin (10 million Unit) was injected to prevent infection. In CAA group, the abdominal aorta was loosely ligated.

Rats of SH and CAA groups were lavaged by physiological saline (1 ml · 100 g⁻¹ · d⁻¹) while rats in CAP group were lavaged with Captopril (1mg · 100g⁻¹ · d⁻¹). 15 rats were killed in each group at the 6th week, the rest rats were killed at the eighth week. There were six rats in SH group, ten in CAA group and eight in CAP group died. The remaining rats were studied at the 8th weeks in each group.

**Hemodynamical measurement**

After intraperitoneal anesthesia, the systolic artery pressure (SAP), diastolic artery pressure (DAP) were recorded via right carotid artery cannulation. Then the catheter was pushed 4.5-5.0 cm further into the left ventricle to record the left ventricular end-diastolic pressure (LVEDP), the maximal change of left ventricular pressure (± dp/dt). Then, the heart was taken immediately by thoracotomy. After both of
the atrium and the right ventricle were cut off, the weight of left ventricle was measured and the left ventricular mass index (LVMI) was calculated. Local myocardium from the apical site was taken for transmission electron microscopy examination. The rest was stored in liquid nitrogen for later use.

Detection of the myocardial energy-rich phosphates

Apical myocardium 200 mg was sheared after ambo-thawing, then pre-cooled perchloric (0.42 mol/L,2ml) was added and the mixedt was homogenated. After ice water bath for 5 min and centrifugal of 4000r/min for 5 min, the supernatant fluid were mixtured with chloroform/ methanol 2:1 mixture (0.2ml) and then oscillated for 1 min to extract liposoluble compounds, these compounds was separated by 4000r/min centrifugal for 5min. Then the supernatant (1.5ml) was titrated with sodium hydroxide to the PH of about 6.5. The supernatant was stored in ice water for 5 min and then centrifugated at 4000r/min for 5 min, the supernatant were filtered and preserved in ice water bath. The content of ATP, ADP and AMP was measured within 8h. All the above operation was performed within an environment of 4℃.

Statistical analysis

SPSS statistical software (version 13.0) was used for statistical analysis. Continuous data were presented as. One-way analysis of variance was used for the statistical analyses at the same time among different groups. Pearson’s t-test was used for different time points comparison within the same group. P values of < 0.05 were considered statistically significant.

Results

Changes of cardiac function and energy-rich phosphates

At 6th and 8th week, in CAA group, the cardiac function parameters (SAP, DAP, LVEDP, LVMI) were increased (all \( P < 0.01 \)) and levels of energy-rich phosphates was decreased \( (P<0.01) \). Compared with that of 6th week, the DAP, LVMI, and -dp/dtmax at 8th week were increased significantly \( (P < 0.05) \).

Compared with CAA group, the parameters of SAP, DAP, LVMI, LVEDP in CAP group at 6th and 8th week were decreased \( (P<0.01) \) and the parameters of +dp/dtmax, dp/dtmax were increased \( (P < 0.01) \). At 6th and 8th week, the levels of energy-rich phosphates in CAP group were increased compared with that of CAA group (all \( P<0.01 \)), but EC was not statistically different \( (P>0.05) \). (Table 1 and Table 2).

At the 8th week, the parameters of SAP, DAP, LVMI were decreased \( (P<0.01) \) and the parameters of +dp/dtmax, and -dp/dtmax were increased \( (P<0.01) \). At the 8th week, levels of energy-rich phosphates (ADP, AMP, TAN) were increased \( (P<0.01) \). But ATP and EC was not statistically different \( (P>0.05) \). (Table 1 and Table 2).

Transmission electron microscopy (TEM) examination

Different myocardial ultrastructure changes such as cardiac muscle fibrils chalasia, ambiguity, alignment disorder, myocardial cells edema:crista mitochondriales disorder or vague; vacuolization; decrease quantity of glycogenomes; lipid droplet increase; sarcoplasmic reticulum expansion and interstitial edema were found in both CAA and CAP groups. In CAA group, these injuries were obviously worse at 8th week than that of 6th week. But, in CAP group, these changes were not observed. (Figure 1).

Discussion

The energy of the heart was mainly provided by oxidative phosphorylation of adenosine triphosphate (ATP) in the mitochondria of myocardial cell after glucose and fatty acids intake, with 60%~80% of the energy comes from the oxidation of fatty acid and only 20%~40% from glucose oxidation. In the setting of heart failure, the ATP enzyme protein in the mitochondrial membrane was damaged and the ability to generate ATP was declined. It has been confirmed that for the failure heart, the sway of energy metabolism from fatty acid oxidation to carbohydrates can improve.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison of the cardiac function parameters between 6th and 8th weeks in different groups</th>
</tr>
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<tbody>
<tr>
<td>Group</td>
<td>n</td>
</tr>
<tr>
<td>--------</td>
<td>---</td>
</tr>
<tr>
<td>SH</td>
<td></td>
</tr>
<tr>
<td>6th week</td>
<td>15</td>
</tr>
<tr>
<td>8th week</td>
<td>19</td>
</tr>
<tr>
<td>CAA</td>
<td></td>
</tr>
<tr>
<td>6th week</td>
<td>15</td>
</tr>
<tr>
<td>8th week</td>
<td>15</td>
</tr>
<tr>
<td>CAP</td>
<td></td>
</tr>
<tr>
<td>6th week</td>
<td>15</td>
</tr>
<tr>
<td>8th week</td>
<td>17</td>
</tr>
</tbody>
</table>

a: \( P<0.05 \); b: \( P<0.01 \); Comparison with SH 6th; c: \( P<0.05 \); d: \( P<0.01 \); Comparison with CAA 6th; e: \( P<0.05 \); f: \( P<0.01 \); Comparison with SH 8th; g: \( P<0.05 \); h: \( P<0.01 \); Comparison with CAA 8th; i: \( P<0.05 \); j: \( P<0.01 \); Comparison with CAP 6th
the efficiency and improve the blood oxygen consumption and cardiac systolic function, and the ameliorate the progression of heart failure. Cardiac energy metabolic disorder and energy-rich phosphate depletion are both the important reasons of myocardial injury, ATP content of myocardial can be used to measure the myocardial cell integrity and the feasibility of functional recovery. In physiology and emergency situations, energy charge (EC) can regulate energy metabolism transiently and reversibly through adeynlate kinase, can effectively evaluate the energy reserves of myocardial cell. So, it is considered the reflection of myocardial cell energy reserves.

Kidney ischemia induced by narrowing the abdominal aorta increase the generation of renin. Renin turns angiotensinogen into angiotensin I and II. Meanwhile, renin stimulate the sympathetic nerve system and increase catecholamine output, leading to increased blood pressure. Abdominal aortic stenosis directly elevate blood pressure and increase the afterload of left ventricle. The left ventricle undergo a time course from concentric hypertrophy to eccentric dilation, that is, heart failure. Angiotensin II (angiotensin II, Ang II) is the most active substance in RAS systems. Angiotensin II, by combining with AT1 receptor, cause vasoconstriction, vascular smooth cell and myocardial cell proliferation, aldosterone secretion, upregulate sympathetic nervous tension, cause inflammation and oxidative stress. On the other hand, angiotensin II, by combining with AT2 receptor, lead to vasodilation and anti-proliferation and promote cell apoptosis. The long-term activation of renin-angiotensin-aldosterone system (RAAS) is the main cause of of progressive deterioration and reshape of heart function.

Table 2  Comparison of the level energy-rich phosphate between 6th and 8th in different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
<th>TAN EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH</td>
<td>6th</td>
<td>0.4596 ± 0.0254</td>
<td>0.8549 ± 0.0385</td>
<td>0.8423 ± 0.0196</td>
<td>2.1567 ± 0.0653</td>
</tr>
<tr>
<td></td>
<td>8th</td>
<td>0.4598 ± 0.0193</td>
<td>0.8566 ± 0.0346</td>
<td>0.8368 ± 0.0206</td>
<td>2.1532 ± 0.0472</td>
</tr>
<tr>
<td>CAA</td>
<td>6th</td>
<td>0.1852 ± 0.0345a</td>
<td>0.3795 ± 0.0504a</td>
<td>0.6839 ± 0.0191b</td>
<td>1.2486 ± 0.0576b</td>
</tr>
<tr>
<td></td>
<td>8th</td>
<td>0.1778 ± 0.0220a</td>
<td>0.3072 ± 0.0362a</td>
<td>0.6540 ± 0.0208a</td>
<td>1.1391 ± 0.0384a</td>
</tr>
<tr>
<td>CAP</td>
<td>6th</td>
<td>0.2871 ± 0.0618ab</td>
<td>0.6784 ± 0.0296cd</td>
<td>1.1837 ± 0.0391ab</td>
<td>2.1491 ± 0.0636ab</td>
</tr>
<tr>
<td></td>
<td>8th</td>
<td>0.3085 ± 0.0566bd</td>
<td>0.7257 ± 0.0439bd</td>
<td>1.2206 ± 0.0421bd</td>
<td>2.2548 ± 0.0692bd</td>
</tr>
</tbody>
</table>

a: P<0.05, b: P<0.01, Comparison with SH 6th; c: P<0.05, d: P<0.01, Comparison with CAA 6th; e: P<0.05, f: P<0.01, Comparison with SH 8th; g: P<0.05, h: P<0.01, Comparison with CAA 8th; i: P<0.05, j: P<0.01, Comparison with CAP 6th.

Fig.1  The morphological change of mitochondria by TEM. A:SH Group at 6th week (× 20 000); B:CAA at 8th week (× 20 000); C:CAP at 6th week (× 2 000); D:CAP at 8th week (× 10 000).
Captopril can reduce the level of Ang II in blood circulation and myocardial tissue, inhibit bradykinin decomposition, reduce metalloprotease activity, inhibit myocardial fibrosis and scarring, improve the metabolism of energy-rich phosphate myocardial infarction. 

Previous studies showed captopril compounds can increase cardiac tissue energy-rich phosphates, mainly to promote the content of the synthesis of adenosine triphosphate. The results confirm the function of myocardia has been improved in the rats after captopril treatment. Levels of cardiac muscle ATP, ADP, AMP, TAN are all increased in comparison with the normal range. Lactic acid isozyme and ADP/ATP carrier concentration decreased obviously to the normal range. Lactic acid isozyme and ADP/ATP carrier concentration changes in heart failure, when using ACE I drugs can be improved after cardiac energy balance. 

The results of this study demonstrate that the treatment of Captopril can significantly improve the myocardial energy metabolism in pressure overload rats and can protect the function of myocardial mitochondria. Captopril can improve cellular membrane edema and maintain the stability of membrane structure. Captopril can wipe out oxygen free radicals and protect vascular endothelial cell function, and protect the structure and function of mitochondria integrity. It has been confirmed that improving mitochondrial function and increase the energy-rich phosphates produce of cardiac muscle cell are the main mechanisms of captopril in improving the myocardial energy metabolism.

The cardiac function was obvious improved and the levels of energy-rich phosphates also improved in CAF group at 8th week compared with 6th indicate that the effect of captopril treatment on cardiac muscle energy metabolism increase with time.

This study provides probed the possible mechanism of ACEI in improving the function of congestive failed heart. Further study on the multi-mechanisms of ACEI is needed to provide useful information to clinical practice.

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**References**