Laboratory Research

Effect of hepatocyte growth factor on left ventricular remodeling after acute myocardial infarction in canine

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Background and objectives To investigate the effect of hepatocyte growth factor (HGF) on left ventricular (LV) remodeling after acute myocardial infarction (AMI). Methods AMI was produced by ligation of proximal left anterior descending coronary artery (LAD) in 12 mongrel canines. These animals were randomized into 2 groups. In HGF group (n=6), canines were injected with pc-DNA3-HGF 1ml (about 300ug) at the margin of infarcted myocardium; in control group (n=6) canines were injected with equal volume of normal saline. Cardiac function and left ventricular remodeling were evaluated with echocardiography at 1, 4, 8 weeks after MI. LV myocardium specimens were obtained at 8 weeks and stained with hematoxylin and eosin for histological examination or with Sirius red to assess the collagen content. Results Compared with control group, LVEF in HGF group was significantly higher at 4 weeks (49.61±6.66 vs 39.84±6.39; P<0.05) and at 8 weeks (51.57±8.53 vs 40.61±7.67; P<0.05) after AMI, while LVESV was significantly lower in HGF group than that in control group at 8 weeks after AMI (18.98±3.47 vs 25.66±5.86; P<0.05). Posterior left ventricular wall thickness decreased significantly from 1 wk to 8 wks after AMI in control group, while remained unchanged in HGF group. Compared with control group, histological examination showed more neovascularization and less scar, and Sirius red staining indicated higher volume of type III collagen (7.10±4.06% vs 3.77±1.09%; P<0.05) and lower collagen I/III ratio value (1.01±0.52 vs 2.94±2.48; P<0.05) in HGF group. Conclusion HGF gene transfer might improve cardiac function and LV remodeling after acute myocardial infarction by stimulating angiogenesis, reducing fibrosis, and reducing myocardial scarring. (J Geriat Cardiol 2006;3(2):112-5.)

Key Words hepatocyte growth factor; myocardial infarction; left ventricular remodeling

Coronary artery disease (CAD) is becoming a leading cause of heart failure in China. In spite of the increasing availability of new treatment strategies such as percutaneous transluminal coronary angioplasty (PTCA), stenting as well as thrombolytic therapy in China, 50% of patients with acute myocardial infarction (AMI) will develop symptomatic heart failure within 5 years after the first onset of AMI. Many laboratory researches have verified that myocardial apoptosis and left ventricular (LV) remodeling play important roles in the development of heart failure in AMI patients. Hepatocyte growth factor (HGF) is a mesenchyme-derived pleiotropic factor that induces angiogenesis, antifibrosis, and antiapoptosis in the myocardium. HGF gene delivery into ischemic myocardium by adenoviral vectors was reported to increase angiogenesis, decrease infarct size, and improve cardiac function in myocardial ischemia animal models. However, short action time in vivo, severe systemic side effects, and unpredictable safety of adenoviral vectors limited its clinical use. Recently, a small observational clinical study indicated that intramuscular injection of naked HGF plasmid might be beneficial for patients with ischemic peripheral arterial disease. In this study, we injected a plasmid with complementary DNA encoding human HGF directly into ischemic myocardium in a canine AMI model, to investigate the effects of HGF on LV remodeling and LV function, and the possible mechanism of early prevention of LV remodeling after AMI.

Materials and methods

HGF plasmid
The plasmid of pc-DNA3-HGF was kindly provided by Professor LS Wang of the Chinese Academy of Military Medical Sciences. The plasmid of pc-DNA3-HGF, transformed into DH-5α, was extracted with large-scale EndoFree plasmid purification kits (Qiagen, based on a modified alkaline lysis procedure). To determine the yield and purification, the plasmid DNA was determined by both UV spectrophotometry at 260 nm and agarose gel electrophoresis.

Animal model
The study protocol was approved by the institutional animal research committee of our hospital. Thirteen adult mongrel dogs (17±2 kg) were anesthetized with 3% sodium pentobarbital administered intravenously. After intubation, the dogs were ventilated with 900C respirator. A median thoracotomy was performed to open the chest, a pericardial cradle was created, and the heart was exposed, the left anterior descending coronary artery proximal to the first diagonal branch was isolated and ligated with a suture. Continuous electrocardiogram was monitored during the surgery. ST segment eleva-
tion in more than two adjacent precordial leads was regarded as success in creating the AMI model.

**HGF gene transfer**

Twelve surviving dogs with successfully induced AMI were randomly assigned to two groups. In the HGF group (n=6), immediately after AMI was generated, 300μg (1 ml) of plasmid with a complementary DNA encoding the gene for pc-DNA3-HGF was injected directly into ten points at the myocardium border between the normal tissue and the infarction area. Six other dogs were injected with similar volume of normal saline in the same procedure as control group.

**Assessment of LV function**

Echocardiography was performed to examine left ventricular ejection fraction (LVEF), left ventricular end-diastolic volume (LVEDV), left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic volume (LVESV), and left ventricular end-systolic diameter (LVESD) at 1 week, 4 weeks, and 8 weeks after AMI.

**Measurement of relative LV weight**

All the dogs were sacrificed 8 weeks after operation. The hearts were removed from the body and blood was washed off repeatedly by ice saline. After the vessels, fats, and other non-myocardial tissues were cut off, the whole heart and LV were weighed. Relative LV weight was calculated according to the following formula:

Relative LV weight=LV weight(g)/body weight(kg).9

**Histopathological analysis and determination of cardiac collagen**

The LV was fixed for 1 week in formalin. LV histological samples were taken from each transverse section, and stained with hematoxylin and eosin and sirius red for histological assessment. Contents of type I and type III collagen were determined by computer image analysis system as previously described.10

**Statistical analysis**

Continuous variables are expressed as mean ± SD. Continuous variables were compared with the t-test or rank-sum test. Intra-group difference was compared with one-way ANOVA. P<0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 11.0 for Windows (SPSS Inc., Chicago, IL, USA).

**Results**

**Effect of HGF gene on canine cardiac function after MI**

Compared with control group, LVEF in HGF group was significantly higher at 4 weeks (49.61±6.66 vs 39.84±6.39; P<0.05) and at 8 weeks (51.57±8.53 vs 40.61±7.67; P<0.05) after AMI while LVESV was significantly lower in HGF group than in control group at 8 weeks after AMI (18.98±3.47 vs 25.66±5.86; P<0.05). LVEF increased while LVESV decreased gradually from 1 wk to 8 weeks after infarction in HGF group. However, the differences were not statistically significant (Table 1).

**Effect of HGF gene on LV remodeling**

There were no significant decrease of PWLVT and IVS in HGF group, while PWLVT decreased significantly in the control group from 1 wk to 8 wks after infarction (P=0.04). The relative weights of heart and LV were lower in HGF group than those in control group, but the differences were not statistically significant (Table 2).

**Histopathological analysis**

The number of myocardial capillaries in the ischemic area increased significantly in HGF group while decreased in control groups after AMI. At the same time, island-form myocardial cells survived in the infarction area and at the junction of necrotic and healthy myocardial tissue was found in the HGF group, whereas significant scar formation was observed in the control group.

Type III collagen in HGF group was significantly higher than in control group (7.10±4.06% vs 3.77±1.09; P<0.05). The ratio of type I/type III collagen was lower in HGF group than that in control group (1.11±0.52 vs 2.94±2.48; P<0.05). There was no difference in type I collagen between the two groups (6.74±2.86% vs 10.7±9.57%; P>0.05) (Table 3).

**Table 1. Effect of HGF gene on canine cardiac function after AMI**

<table>
<thead>
<tr>
<th></th>
<th>HGF group</th>
<th>Control group</th>
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<tbody>
<tr>
<td></td>
<td>1 wk</td>
<td>4 wks</td>
</tr>
<tr>
<td>LVEDV</td>
<td>38.49±7.41</td>
<td>39.28±8.48</td>
</tr>
<tr>
<td>LVEDD</td>
<td>3.50±0.37</td>
<td>3.61±0.28</td>
</tr>
<tr>
<td>LVESD</td>
<td>2.65±0.32</td>
<td>2.84±0.29</td>
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<tr>
<td>LVEF</td>
<td>43.13±8.88</td>
<td>49.61±6.66*</td>
</tr>
</tbody>
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*P<0.05, compared with control group. LVEDV: left ventricular end-diastolic volume; LVESV: left ventricular end-systolic volume; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; LVEF: left ventricular ejection fraction.
Table 2. Effect of HGF gene on LV remodeling

<table>
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<tr>
<th></th>
<th>HGF group</th>
<th>Control group</th>
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<tbody>
<tr>
<td></td>
<td>1 wk 4 wk 8 wk</td>
<td>1 wk 4 wk 8 wk</td>
</tr>
<tr>
<td>PWLVT(cm)</td>
<td>0.72±0.12*</td>
<td>0.73±0.08</td>
</tr>
<tr>
<td>IVST(cm)</td>
<td>0.75±0.13</td>
<td>0.79±0.14</td>
</tr>
<tr>
<td>HRW(g/kg)</td>
<td></td>
<td>6.86±0.90</td>
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<tr>
<td>LVRW(g/kg)</td>
<td></td>
<td>5.02±0.34</td>
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* P<0.05, compared with control group

Table 3. Effect of HGF gene on collagen composition

<table>
<thead>
<tr>
<th></th>
<th>HGF group</th>
<th>Control group</th>
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<tr>
<td></td>
<td>type I collagen</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.74±2.86</td>
<td>10.7±9.57</td>
</tr>
<tr>
<td></td>
<td>type III collagen</td>
<td>7.10±4.06*</td>
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<tr>
<td></td>
<td>type I to type III ratio</td>
<td>1.11±0.52*</td>
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</table>

* P<0.05, compared with control group

Discussion

Left ventricular (LV) enlargement is common after MI. This process, termed postinfarction ventricular remodeling, is associated with heart failure and increased mortality. Ventricular remodeling is known to be affected by several factors. One major factor is the infarct size, which can be limited by early opening the infarct-related artery (early reperfusion) or by the presence of collateral vessels. Another major factor is scar formation in the infarcted region, which is related with abnormal deposit of collagen. Previous studies have shown that HGF have antiapoptotic, angiogenic, and antifibrotic effects in the ischemic myocardium. Therefore, gene therapy with HGF-complementary DNA plasmids may enhance the chance of "bridge to recovery." On the other hand, administration of human recombinant HGF prevented and/or reversed fibrotic process in the myocardium by stimulating the degradation of extracellular matrix and inhibiting its synthesis.

Soeki et al. found that serum HGF concentration on day 7 after AMI was mostly from noncardiac sources and could predict the level of left ventricular remodeling. Our study demonstrated that injection of HGF plasmid into myocardium at the acute phase after AMI could improve LV systolic function and significantly decrease adverse LV remodeling. These effects may be associated with many mechanisms, including (1) HGF promotes blood capillary regeneration, increases collateral vessels, improves blood supply of myocardium, and reduces formation of scar tissue and ventricular aneurysm; (2) HGF may relieve collagen deposition, decrease stiffness of LV, prevent LV wall thinning, leading to decrease of myocardium oxygen consumption and increase of LV contraction. Songet al. found that intravenous injection of HGF could decrease myocardial cell apoptosis, then improve ventricular remodeling. Taniyama et al. showed that the increase of local HGF expression can decrease myocardial fibrosis by inhibiting angiotensin II production. These two studies were in agreement with our current results.

In summary, our results showed that direct injection of plasmid with HGF gene could improve cardiac function and LV remodeling after acute myocardial infarction. This effect might be mediated by the angiogenic and antifibrotic effect of HGF.

References


