Concentrations of Hepatocyte Growth Factor, Basic Fibroblast Growth Factor, and Vascular Endothelial Growth Factor in Pericardial Fluid and Plasma

Tetsuya KUBOTA,1 MD, Atsushi NAMIKI,1 MD, Masayuki FUKAZAWA,1 MD, Michiro ISHIKAWA,1 MD, Masao MOROI,1 MD, Kunio EBINE,2 MD, and Tetsu YAMAGUCHI,1,3 MD

SUMMARY

Some angiogenic factors, including hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF), have been reported to promote angiogenesis and improve myocardial perfusion in experimental models of ischemic heart disease. These factors are produced in various tissues, including myocardium. We measured the concentrations of HGF, bFGF, and VEGF by enzyme-linked immunosorbent assay in plasma and in pericardial fluid sampled during open heart surgery (12 patients with ischemic heart disease and 17 with nonischemic heart disease). HGF levels were significantly higher in plasma than in pericardial fluid (12.0 ± 1.8 versus 0.26 ± 0.04 ng/mL, P < 0.0001). On the other hand, bFGF levels were significantly higher in pericardial fluid than in plasma (243.5 ± 50.9 versus 49.6 ± 7.8 pg/mL, P = 0.009). VEGF levels were not significantly different between pericardial fluid and plasma (47.2 ± 17.6 versus 24.5 ± 3.6 pg/mL, P = 0.23). Concentrations of angiogenic factors in pericardial fluid and in plasma were not significantly different between patients with ischemic and nonischemic heart disease. These results suggest that the production, secretion, and kinetics of HGF, bFGF, and VEGF are different. These angiogenic factors may have different pathophysiologic roles. (Jpn Heart J 2004; 45: 989-998)

Key words: Hepatocyte growth factor, Basic fibroblast growth factor, Vascular endothelial growth factor, Pericardial fluid, Ischemic heart disease, Nonischemic heart disease

ANGIOGENESIS is induced by hypoxia, and has an important physiologic role in ischemic tissue.1) Numerous growth factors have been reported to induce angiogenesis.2) Hepatocyte growth factor (HGF) is a mesenchyme-derived pleiotropic factor and one of the growth factors which proliferate endothelial cells.3) Basic fibroblast growth factor (bFGF) is a heparin-binding protein which is mitogenic for endothelial cells and smooth muscle cells.4) Vascular endothelial growth...
factor (VEGF) is a heparin-binding glycoprotein and endothelial cell specific mitogen. These angiogenic factors play important roles in the pathophysiology of cardiovascular conditions, including the development of atherosclerosis and restenosis after coronary angioplasty. Preclinical studies have documented that these angiogenic factors promote angiogenesis and improve blood flow in animal models of either limb or myocardial ischemia. Recently, preliminary clinical trials have been performed in patients with limb or myocardial ischemia.

HGF, bFGF, and VEGF have been isolated from various tissues, including human cardiac tissues and may be present in pericardial fluid. Pericardial fluid is an ultrafiltrate of plasma, but also contains substances produced in and secreted from the myocardium. The production and release of angiogenic growth factors in normal and diseased myocardium can affect their concentrations in pericardial fluid. These angiogenic factors, contained in pericardial fluid, may play an important role in the development of collateral vessels in ischemic heart tissue. However, the pathophysiologic roles of angiogenic growth factors in pericardial fluid have not been investigated fully and have not been evaluated comparatively. To investigate the pathophysiologic role of angiogenic growth factors, we measured the concentrations of HGF, bFGF, and VEGF in pericardial fluid and plasma in patients with ischemic or nonischemic heart disease using enzyme-linked immunosorbent assays (ELISA).

**METHODS**

**Patients:** The 29 patients in this study underwent open heart surgery for valvular disease, coronary artery disease, congenital heart disease, or aortic aneurysm. Twelve patients had ischemic heart disease (7 stable effort angina pectoris, 5 unstable angina pectoris) and 17 patients had nonischemic heart disease (7 mitral valve regurgitation, 2 mitral stenosis, 4 aortic regurgitation, 1 aortic stenosis, 2 atrial septal defect, 1 thoracic aortic aneurysm). The clinical diagnosis of coronary artery disease was verified by coronary angiography.

**Sampling and measurements:** Samples of pericardial fluid were obtained immediately after incision of the pericardium (before heparinization). Blood samples were withdrawn from the cannulated brachial artery at the same time. The samples were collected in tubes (7 mL) containing EDTA-2Na (10.5 mg), centrifuged at 3000 g for 10 minutes at 4°C, and frozen at -80°C. HGF concentrations were measured using an ELISA kit (Quantikine, R&D Systems) by a quantitative sandwich enzyme immunoassay technique. Monoclonal antibodies specific for HGF had been coated onto the microtiter plate provided in the kit. Standards and samples were pipetted into the wells and any HGF present was bound by the immobilized antibodies. After washing away any unbound proteins, an enzyme-linked
A polyclonal antibody specific for HGF was added. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells. After an incubation period, an amplifier solution was added to the wells, allowing color to develop in proportion to the amount of HGF in each sample. The color development was stopped and the color intensity was measured. Concentrations of bFGF and VEGF were measured using ELISA kits (bFGF, Quantikine, R&D Systems; VEGF, BIOTRAK, Amersham LIFE SCIENCE).

**Statistical analysis:** All values are expressed as the mean ± SEM. Differences were analyzed with Student’s t test. P values < 0.05 were considered statistically significant.

## RESULTS

**Clinical characteristics of the patients:** The mean age of patients in this study (n = 29; 17 males and 12 females) was 61 ± 2 years. The average systolic and diastolic blood pressures were 131 ± 4 and 79 ± 3 mm Hg, respectively. Mean heart rate was 78 ± 3/min. Echocardiographic examinations revealed mean left ventricular end-diastolic and end-systolic diameters and left ventricular ejection fractions of 54 ± 2 mm, 37 ± 2 mm, and 62 ± 3%, respectively. The table shows the

<table>
<thead>
<tr>
<th></th>
<th>IHD (n = 12)</th>
<th>NIHD (n = 17)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63 ± 3</td>
<td>60 ± 2</td>
<td>0.49</td>
</tr>
<tr>
<td>Male/female</td>
<td>9/3</td>
<td>8/9</td>
<td>0.14</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>140 ± 6</td>
<td>124 ± 4</td>
<td>0.04</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>85 ± 4</td>
<td>73 ± 3</td>
<td>0.04</td>
</tr>
<tr>
<td>Heart rate (/min)</td>
<td>77 ± 4</td>
<td>77 ± 3</td>
<td>0.95</td>
</tr>
<tr>
<td>Echocardiogram</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVDd (mm)</td>
<td>52 ± 3</td>
<td>55 ± 2</td>
<td>0.84</td>
</tr>
<tr>
<td>LVDs (mm)</td>
<td>36 ± 4</td>
<td>37 ± 2</td>
<td>0.54</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>59 ± 5</td>
<td>63 ± 3</td>
<td>0.39</td>
</tr>
<tr>
<td>Medication (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>41</td>
<td>47</td>
<td>0.82</td>
</tr>
<tr>
<td>β-Blocker</td>
<td>33</td>
<td>0</td>
<td>0.05</td>
</tr>
<tr>
<td>Antiplatelet</td>
<td>75</td>
<td>23</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Diuretic</td>
<td>0</td>
<td>76</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Nitrate</td>
<td>58</td>
<td>11</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Calcium antagonist</td>
<td>41</td>
<td>35</td>
<td>0.69</td>
</tr>
</tbody>
</table>

IHD = ischemic heart disease; NIHD = nonischemic heart disease; BP = blood pressure; LVDd = left ventricular end-diastolic diameter; LVDs = left ventricular end-systolic diameter; LVEF = left ventricular ejection fraction; ACE = angiotensin converting enzyme.
differences in clinical characteristics between patients with ischemic and nonischemic heart disease. The two groups (ischemic and nonischemic heart disease) did not show significant differences in age, gender distribution, heart rate, left ventricular end-diastolic diameters, left ventricular end-systolic diameters, or left ventricular ejection fraction. Echocardiographic examinations revealed that there was no difference in interventricular septal wall thickness or left ventricular posterior wall thickness (data not shown).

Figure 1. Concentrations of HGF, bFGF, and VEGF in pericardial fluid and in plasma of all patients (n = 29). Values are expressed as the mean ± SEM. HGF = hepatocyte growth factor; bFGF = basic fibroblast growth factor; VEGF = vascular endothelial growth factor.
Concentrations of HGF, bFGF, and VEGF in pericardial fluid and in plasma of all patients: Figure 1 shows that the HGF levels of all patients were significantly higher in plasma than in pericardial fluid (12.0 ± 1.8 versus 0.26 ± 0.04 ng/mL, \( P < 0.0001 \)). Moreover, HGF concentrations in pericardial fluid correlated with plasma HGF concentrations (data not shown). On the other hand, bFGF levels in all patients were significantly higher in pericardial fluid than in plasma (243.5 ± 50.9 versus 49.6 ± 7.8 pg/mL, \( P = 0.009 \); Figure 1). No significant correlation was found between bFGF concentrations in pericardial fluid and plasma. VEGF levels in all patients were not significantly different between plasma and pericardial fluid (47.2 ± 17.6 versus 24.5 ± 3.6 pg/mL, \( P = 0.23 \); Figure 1). HGF levels were significantly higher in plasma than in pericardial fluid in patients with ischemic heart disease and in those with nonischemic heart disease (0.23 ± 0.19 ng/mL in pericardial fluid versus 13.6 ± 2.3 ng/mL in plasma in patients with ischemic heart disease, \( P = 0.0001 \); and 0.29 ± 0.07 versus 10.9 ± 2.6 ng/mL in patients with nonischemic heart disease, \( P = 0.001 \), respectively). bFGF levels were significantly higher in pericardial fluid than in plasma in both patients with ischemic heart disease and with nonischemic heart disease, and the VEGF levels of patients with not only ischemic but also nonischemic heart disease were not significantly different between plasma and pericardial fluid, like those of all patients (data not shown).

Concentrations of HGF, bFGF, and VEGF in patients with ischemic and nonischemic heart disease in pericardial fluid and in plasma: As shown in Figure 2, HGF concentrations in patients with ischemic and nonischemic heart disease were not significantly different not only in pericardial fluid but also in plasma. The bFGF and VEGF levels in patients with ischemic and nonischemic heart disease also showed no significant difference in pericardial fluid and in plasma. HGF in plasma, bFGF in pericardial fluid, and VEGF of patients with nonischemic heart disease showed various levels. We could not clarify the relationship between background disease and each angiogenic factor level in pericardial fluid or in plasma.
Figure 2. Concentrations of HGF, bFGF, and VEGF in patients with ischemic and nonischemic heart disease in pericardial fluid and in plasma. Values are expressed as the mean ± SEM. HGF = hepatocyte growth factor; bFGF = basic fibroblast growth factor; VEGF = vascular endothelial growth factor.
DISCUSSION

It has been reported that in patients with acute myocardial infarction, circulating HGF levels start to elevate within 3 hours after onset. The sources of circulating HGF are unclear, but a recent report showed that cardiac HGF secretion remained increased up to approximately 4 weeks after myocardial infarction. On the other hand, it also has been reported that the HGF gene is downregulated by hypoxia. As pericardial fluid contains substances produced and secreted by the myocardium, we examined whether HGF levels in pericardial fluid are increased in patients with ischemic heart disease. This is the first report investigating the concentration of HGF in pericardial fluid. In patients with ischemic heart disease, as well as in those with nonischemic heart disease, HGF levels were significantly higher in plasma than in pericardial fluid. Moreover, HGF levels in pericardial fluid were not significantly different between patients with ischemic and nonischemic heart disease. Although we have no data for a healthy control population, chronic ischemia may have no effect on HGF levels.

bFGF levels were significantly higher in pericardial fluid than in plasma. This result is consistent with the findings of Corda, et al who suggested that bFGF in pericardial fluid might participate in the development of ventricular hypertrophy. It has been reported that bFGF levels in the pericardial fluid of patients with unstable angina are significantly higher than in patients with nonischemic heart disease, and that bFGF in pericardial fluid may accelerate the growth of human vascular smooth muscle cells, induce angiogenesis, and increase collateral circulation. Uchida, et al have reported that bFGF administered into the pericardial space promotes angiogenesis and reduces infarction size in dogs with acute myocardial infarction. bFGF levels in pericardial fluid were not significantly different between patients with ischemic and nonischemic heart disease in this study. This finding may be due to the timing and grade of myocardial ischemia, since there were no patients with acute myocardial infarction in this study. Serum bFGF levels in patients with acute myocardial infarction have been reported to be elevated. Our results indicate that bFGF levels in pericardial fluid, which reflect production and secretion by the myocardium, do not differ between patients with ischemic and nonischemic heart disease.

VEGF is known to be a potent endothelial cell-specific mitogen. It has been reported that serum VEGF levels reflect production by peripheral blood mononuclear cells in patients with acute myocardial infarction, and are maximally elevated on day 7. In this study, VEGF concentrations in pericardial fluid were not significantly different between patients with ischemic and nonischemic heart disease. VEGF levels were not significantly different in plasma and pericardial fluid. These results may indicate that VEGF is produced and secreted by the
myocardium in nonischemic heart tissue to the same extent as in chronically ischemic heart tissue. Shinohara, et al\textsuperscript{19} reported that VEGF mRNA was found not only in ischemic human cardiomyocytes but also nonischemic cardiomyocytes, which supports this hypothesis. VEGF in plasma may be derived from peripheral blood mononuclear cells and vascular smooth muscle cells, and VEGF in pericardial fluid may be produced by cardiomyocytes. Fujita, et al\textsuperscript{29} reported that the level of VEGF in pericardial fluid was increased only in patients with severe rest angina within 2 days before emergency coronary artery bypass graft surgery. They showed that the level of VEGF in pericardial fluid in patients with stable angina was similar to that of nonischemic heart disease patients. In our study, there were no patients with severe acute myocardial ischemia including acute myocardial infarction. We observed similar results to those reported by Fujita, et al in stable chronic myocardial ischemic patients.

It has been reported that both pericardial fluid and serum increased the rate of protein synthesis, and the magnitude of change with pericardial fluid was 3-fold greater than with serum.\textsuperscript{24} These trophic effects of pericardial fluid were inhibited by anti-bFGF antibodies. However, the bFGF concentration in pericardial fluid was not correlated to the patient's left ventricular mass. These results suggest that bFGF in pericardial fluid may be a major determining factor in normal myocyte growth, whereas it is not the definitive factor for left ventricular hypertrophy. We did not investigate the effects of angiogenic factors in pericardial fluid on the myocardium or collateral coronary circulation. The role of angiogenic factors in pericardial fluid is important. If angiogenic factors in pericardial fluid are only the result of production and secretion by the myocardium, their presence would have limited clinical therapeutic implications. If angiogenic factors in pericardial fluid affect the growth and vasculature of the myocardium, administration of these factors to the pericardial space might be applied for therapeutic use. In this study, we examined the differences in pericardial fluid and plasma concentrations of HGF, bFGF, and VEGF. Further investigation is needed to clarify the role of angiogenic factors in pericardial fluid and plasma.

**Study limitations:** There are some limitations to this study. First, there were no patients with acute myocardial infarction. It is possible that there are differences in the concentrations of angiogenic factors in pericardial fluid and plasma in patients with acute and chronic ischemia. Second, the cardiac function of the patients in this study was only mildly impaired (mean left ventricular ejection fraction, 62\%), allowing these patients to be candidates for open heart surgery. It is possible that cardiac function affects the concentrations of angiogenic factors in pericardial fluid and plasma. Third, the secretion and absorption of pericardial fluid is not understood completely. Therefore, the proportions of HGF, bFGF, and VEGF in pericardial fluid which are derived from the myocardium are unknown.
Finally, some medications may alter the production and secretion of angiogenic growth factors. Angiotensin converting enzyme inhibitors, for example, may affect HGF production.\(^{30}\) These agents, however, were administered equally to ischemic and nonischemic heart disease patients.

**Conclusions:** We have demonstrated that HGF levels in plasma are higher than those in pericardial fluid, bFGF levels in pericardial fluid are higher than those in plasma, and VEGF levels are not significantly different in pericardial fluid and plasma. There were no differences in the HGF, bFGF, and VEGF levels in pericardial fluid and plasma between patients with ischemic and nonischemic heart disease. These results suggest that HGF, bFGF, and VEGF, which may play important roles in developing collateral vessels in the myocardium, may have different patterns of production, secretion, and kinetics and different pathophysiological roles.

**ACKNOWLEDGEMENT**

We thank Mr. Kazuhiro Kunimi for his excellent technical assistance.

**REFERENCES**

1. Adair TH, Gay WJ, Montani JP. Growth regulation of the vascular system: evidence for a metabolic hypoth-
3. Matsumoto K, Nakamura T. Emerging multipotent aspects of hepatocyte growth factor. J Biochem 1996; 119: 
    591-600. (Review)
5. Ferrara N, Henzel W. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascu-
    endothelial growth factor on coronary collateral development and the arterial response to injury. Circulation 
    1996; 147: 1649-60.
    lial growth factor augments revascularization in a rabbit ischemic hind limb model. J Clin Invest 1994; 93: 662-
    70.
    endothelial growth factor augments collateral development and tissue perfusion. Circulation 1996; 94: 3281-
    90.
11. Yang HT, Deschenes MR, Ogilvie RW, Terjung RL. Basic fibroblast growth factor increases collateral blood 


