Expression of Vascular Endothelial Growth Factor in Patients With Acute Myocardial Infarction

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OBJECTIVE
The purpose of this study was to investigate the clinical significance of vascular endothelial growth factor (VEGF) in acute myocardial infarction (AMI). We also examined the involvement of peripheral blood mononuclear cells (PBMCs), which are a possible source of VEGF in AMI.

BACKGROUND
VEGF is a potent endothelial cell–specific mitogen and could affect the outcome of AMI.

METHODS
Thirty patients with AMI were used for this study. Serum and PBMCs were isolated from peripheral blood on days 1, 7, 14 and 21 after the onset of AMI. PBMCs were cultured at a density of $5 \times 10^6$ cells/ml for 24 h. VEGF levels in serum and the culture media were measured by enzyme-linked immunosorbent assay using a specific anti-human VEGF antibody.

RESULTS
Serum VEGF levels elevated gradually after the onset of AMI and reached a peak on day 14. VEGF levels in the culture medium of PBMCs after incubation for 24 h (PBMC-VEGF) were maximally elevated 7 days after the onset. Maximum serum VEGF levels showed significant positive correlations with maximum creatine phosphokinase (CPK) levels ($r = +0.70$, $p < 0.001$), but maximum PBMC-VEGF levels did not correlate with maximum CPK levels. Patients showing improvement in left ventricular systolic function during the course of AMI showed significantly higher PBMC-VEGF levels than patients without improvement.

CONCLUSIONS
The extent of myocardial damage contributes to the elevation of serum VEGF levels in AMI. VEGF produced by PBMCs may play an important role in the improvement of left ventricular function by promoting angiogenesis and reendothelialization after AMI. (J Am Coll Cardiol 2000;35:968–73) © 2000 by the American College of Cardiology

Growth factors play important roles in the pathogenesis of cardiovascular diseases, such as the development of atherosclerosis and restenosis after coronary angioplasty, by regulating cellular proliferation, migration, differentiation and apoptosis (1,2). Vascular endothelial growth factor (VEGF) is a potent mitogen for endothelial cells. VEGF has been reported to promote collateral formation in ischemic cardiac muscles (3–7) and tissue repair after wounding (8). In animal models, application of recombinant VEGF to ischemic limbs induced angiogenesis and improved tissue perfusion (9). Recently, Baumgartner et al. (10) demonstrated that intramuscular injection of naked plasmid DNA encoding VEGF improved limb ischemia by promoting angiogenesis in patients with critical peripheral arterial disease.

Previously, Seko et al. (11) found that patients with acute myocardial infarction (AMI) have elevated circulating VEGF levels compared with normal subjects. However, the mechanisms and clinical significance of VEGF during AMI are not fully understood. The course of AMI is a complex, dynamic process involving not only myocardial cells but also other cells, such as vascular smooth muscle cells (VSMCs), endothelial cells and circulating blood cells. Various types of cells, including cardiocytes and VSMCs, have been shown to be synthesis sites for VEGF. In addition, blood cells such as megakaryocytes (12), lymphocytes, macrophages (13) and neutrophils (14) can secrete VEGF. In the present study, we investigated the clinical significance of VEGF in patients with AMI. We measured serum VEGF levels in these patients and also focused on the role of peripheral blood
mononuclear cells (PBMCs), which are one of the most important sources of VEGF.

**METHODS**

**Patients.** We studied 30 AMI patients (25 males and 5 females, aged 61.6 ± 10.7 years, ranging from 40 to 80 years) admitted to Jichi Medical School Hospital from August 1997 to July 1998. Diagnosis of AMI was based on the presence of ST segment elevation on the electrocardiogram and a plasma creatine phosphokinase (CPK) level more than double the normal value. We also studied 12 healthy subjects as controls (nine males and three females, aged 55.3 ± 4.7 years, ranging from 34 to 72 years). All patients, with proper indications, received emergent coronary angiography (CAG) and primary percutaneous transluminal coronary angioplasty (PTCA). Left ventriculography (LVG) was performed immediately after primary PTCA. LVG was not performed in patients with an increased plasma creatinine level (from 2.0 to 2.5 mg/dL). Heparin and nitroglycerin were administered intravenously during PTCA. Administration of heparin was continued for one to two days after admission. Aspirin was given to all patients. The use of β-blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors and nitrate preparations was decided by the attending physician. None of the AMI patients had severe heart failure, hepatic failure, renal failure or apparent infectious disease. Re-CAG and LVG were performed before discharge. The left ventricular ejection fraction (LVEF) was evaluated by LVG using an area-length method (15).

**Blood collection.** In our preliminary study, systemic administration of heparin markedly decreased circulating VEGF levels, but this effect was transient and normal levels were recovered 24 h after heparin administration. We also found that VEGF levels in plasma isolated using heparin as an anticoagulant were underestimated (10.0 ± 2.5%) compared with those in serum or plasma isolated without heparin. Thus, all blood samples were obtained under conditions without using heparin.

All patients gave written informed consent. Peripheral blood was drawn from patients at the time of admission and before administration of heparin (day 1). Peripheral blood was also obtained 7, 14 and 21 days after the onset of AMI. At the time of blood sampling, heparin were not administered to any patients. Serum was obtained by centrifugation and stored at −80°C until being assayed for VEGF levels. Attending doctors also obtained blood samples separately at the time of admission and every 4 h until the maximum CPK level was determined.

**Isolation of PBMCs.** Anticoagulated peripheral blood (20 ml total, containing 0.5 ml heparin) was obtained from patients with AMI on day 1 (the time of admission), 7, 14 and 21 days after the onset and also from normal control subjects. The blood was layered onto Mono-Poly resolving medium (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan), and centrifuged at 400g for 20 min at room temperature. The PBMC fraction was drawn up and rinsed with phosphate-buffered saline (0.01 mol/liter sodium phosphate, 0.14 mol/liter NaCl, pH 7.2). After it was centrifuged again at 400g for 12 min at room temperature, the cells were resuspended in 1 ml of RPMI medium containing 10% FBS. The PBMCs were cultured in the same medium at a density of 5 × 10⁶ cells/ml in a humidified atmosphere of 5% CO₂ at 37°C. The supernatants were collected 6, 12 and 24 h after incubation and stored at −80°C until being assayed.

**VEGF assay.** VEGF concentrations in serum and the culture media were determined using a specific enzyme-linked immunosorbent assay (ELISA) kit (Amersham, International, Buckinghamshire, UK). Measurements were performed according to the manufacturer's instructions. The standard curve was linear from 15.6 to 1,000 pg/ml of VEGF.

**Statistical analysis.** Data are expressed as means ± SEM. Comparisons of VEGF levels between the patients and control subjects were analyzed by repeated-measures analysis of variance followed by Scheffe’s test. VEGF levels between the patients with and without improvement in LVEF were compared by Student’s unpaired t test. A simple linear regression line was calculated by the least square method for assessment of the correlations between two parameters. Values of p < 0.05 were considered statistically significant.

**RESULTS**

**Basic characteristics of patients.** Of the 30 patients, 10 had anteroseptal, 18 inferior and 2 lateral AMI. No patients had any previous myocardial infarction. Fourteen patients had hypertension (47%), 15 hyperlipidemia (50%), and 6 diabetes mellitus (20%). Twenty-six patients were smokers (87%), and 10 had a family history of coronary artery disease (33%). The mean time from the onset of symptoms to admission was 6.8 ± 1.3 h, with a range of 1 to 24 h. Emergent CAG was performed on all patients. Of the 30 patients, spontaneous recanalization of the infarction-
related artery was recognized in eight patients. PTCA was performed on 27 patients. Re-CAG was performed on all patients and revealed that patency of dilated vessels was preserved in all patients. The mean of maximum plasma CPK activity was $3,193 \pm 364$ IU/liter, ranging from 371 to 8,642 IU/liter.

Serum VEGF levels. Figure 1 shows changes in serum VEGF levels in patients with AMI from one to 21 days after onset. Serum VEGF levels in AMI patients showed a peak elevation on day 14 ($163 \pm 26.2$ pg/ml), which was significantly higher than the control subjects ($68.7 \pm 16.4$ pg/ml). Solid circles: mean serum VEGF levels in AMI patients; open circles: mean serum VEGF levels in control subjects. Values are expressed as mean $\pm$ SEM. *p < 0.05 compared with control subjects.

VEGF production by PBMCs. Peripheral blood was drawn from patients on days 1, 7, 14 and 21 after the onset of AMI for isolation of PBMCs. Figure 2 shows VEGF levels in the supernatant of cultured PBMCs from the patients on day 7 after the onset of AMI and those from control subjects. VEGF levels in the supernatant of cultured PBMCs from the patients with AMI increased with incubation time, and were significantly higher than those of the control subjects after incubation for 12 and 24 h. We defined the VEGF level in the supernatant of cultured PBMCs after incubation for 24 h as “PBMC-VEGF level” and used this as a marker of the VEGF production ability of PBMCs.

Figure 3 shows changes in PBMC-VEGF levels in the course of AMI. PBMC-VEGF levels in the patients were significantly higher than those in control subjects ($129 \pm 23.1$ pg/ml) from 7 to 14 days after onset of AMI, with a peak on day 7 after onset of AMI ($344 \pm 63.5$ pg/ml).
Correlations between VEGF levels and laboratory data. We then analyzed the correlation between serum VEGF levels and clinical parameters in AMI. As shown in Figure 4A, a significant positive correlation was found between maximum serum VEGF levels and maximum CPK levels (\( r = +0.64, p < 0.001 \)). Similarly, a significant positive correlation was found between maximum CPK-MB and maximum serum VEGF levels (\( r = +0.62, p < 0.001 \)). Significant positive correlations were also observed between maximum CPK and serum VEGF levels on day 7 (\( r = +0.54, p < 0.01 \)) and those on day 14 (\( r = +0.58, p < 0.001 \)). On the other hand, no significant correlations were found between maximum PBMC-VEGF and maximum CPK levels (Fig. 4B).

Correlations between VEGF levels and laboratory data. A significant positive correlation was found between maximum serum VEGF levels and maximum CPK levels in the course of AMI (\( r = +0.64, p < 0.001 \)). Patients receiving heparin for over seven days were excluded because maximum serum VEGF levels can be underestimated in such patients. B, No significant correlation was found between maximum PBMC-VEGF levels and maximum CPK levels.

DISCUSSION

The present study revealed significant elevation of serum VEGF levels and VEGF production by PBMCs in patients with AMI, compared with normal subjects during the acute and subacute phases of myocardial infarction. A significant positive correlation between maximum serum VEGF and CPK-MB levels suggests that the extent of myocardial infarction is linked to the elevation of circulating VEGF.
levels. Similar to our results, Kawamoto et al. (16) reported that serum VEGF levels were significantly associated with peak CPK levels in patients with AMI. Previously, Li et al. (17) reported a sustained increase in VEGF mRNA expression after AMI in the rat model. They observed a marked increase in VEGF mRNA expression in microvessels at the infarction edge or new vessels developed in infarcted myocardium from 24 h to six weeks after infarction. Several cytokines such as interleukin (IL)-6 and IL-8 have been reported to be elevated in the acute stage of AMI (18–28). These cytokines regulate expression of various growth factors. Dembinska-Kiec et al. (29) reported that IL-1β enhances VEGF expression in VSMCs, and Brogi et al. (30) demonstrated that transforming growth factor-β1 and platelet-derived growth factor upregulate VEGF expression in VSMCs. Maximum elevation of serum VEGF levels were observed on day 14 after onset. It is speculated that cytokine-mediated mechanisms are at least partially responsible for the induction of VEGF production in the subacute phase of AMI.

We also examined VEGF production by PBMCs isolated from patients with AMI, because PBMCs are an important source of VEGF (13). Production of VEGF by PBMCs in patients with AMI from seven to 14 days after the onset was significantly higher than that of normal subjects. A weak positive correlation was observed between PBMC-VEGF and serum VEGF levels in patients with AMI only on day 7 after the onset, suggesting that VEGF produced by PBMCs does not strongly contribute to circulating VEGF levels. We could not find a significant correlation between PBMC-VEGF levels and clinical parameters, such as maximum CPK levels. The regulatory mechanisms responsible for enhanced VEGF production by PBMCs after AMI are still unclear. We should also clarify types of mononuclear cells that are mainly involved in enhanced VEGF production.

We found that patients with improvement in left ventricular systolic function had higher maximum PBMC-VEGF levels than those without improvement. Shinozaki et al. (31) reported increased expression of VEGF mRNA in macrophages around the site of infarcted myocardium in an autopsy case of AMI, which was not found in hearts with old myocardial infarctions. Pearlman et al. (32) demonstrated that direct infusion of recombinant human VEGF improved global LVEF and regional wall motion after AMI. We only measured VEGF production by peripheral PBMCs, but if they represent PBMCs infiltrated in the infarcted myocardium, our data suggest that local VEGF produced by PBMCs in the heart may play an important role in the restoration of ventricular systolic function after AMI. Our results also suggest that PBMCs are more closely involved in the improvement in left ventricular systolic function after AMI than circulating VEGF. We could not find any angiographical development of major collateral vessels after AMI because the patency of dilated lesions was preserved in all patients. It is speculated that enhanced VEGF production by locally infiltrated PBMCs in the infarcted myocardium promotes proliferation of endothelial cells and development of microvessels, leading to healing of the infarcted myocardium and repair of the injured endothelium.

Study limitation. In our study, maximum CPK values did not always reflect infarction size because reperfusion of the occluded coronary artery occurred at different time points after the onset of ischemia. Further studies using parameters that reflect infarction size more precisely should be considered. In addition, only patients who did not receive immediate heparin therapy were included in the present study, and because the study population had mean LVEF > 50% at the time of admission and before discharge, our study did not assess serum VEGF and PBMC-VEGF levels in certain subgroups of AMI patients, such as those with large
infarction and poor LV function. Further studies will have to address these subpopulations of AMI patients to assess the relationship between serum VEGF and PBMC-VEGF levels and the outcome.

Conclusions. Increased serum VEGF levels and enhanced production of VEGF by PBMCs were found in patients with AMI. Elevation of circulating VEGF levels may have a cardiovascular protective effect by promoting angiogenesis and proliferation of endothelial cells. PBMCs do not strongly contribute to the elevation of circulating VEGF levels, but may have an important role in the improvement of ventricular function by enhancing local VEGF production in the heart during the subacute phase of AMI.

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