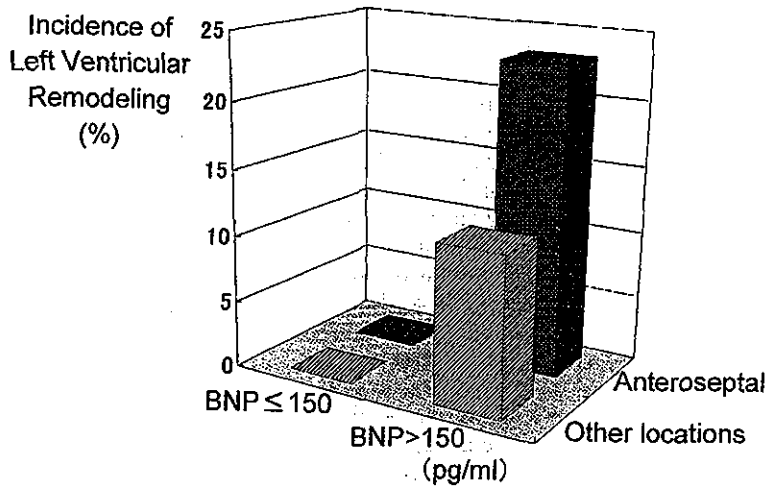

Fig 2. Incidence of left ventricular remodeling according to plasma BNP concentrations and infarct location. The bars indicate the percentage of patients who showed left ventricular remodeling (delta-LVDd >5 mm) in each category. The incidence of left ventricular remodeling was 23.1% in the category of anteroseptal infarction and BNP >150 pg/ml, 11.8% in the category of other locations and BNP >150 pg/ml, and 0% in the two categories with BNP ≤150 pg/ml regardless of infarct location.

centration >500 pg/ml had delta-LVDd >5 mm at follow-up, suggesting that patients with both of these factors may be at high risk of LV remodeling.

The 2 patients who showed definite ischemic ECG changes in the initial exercise test had delta-LVDd <5 mm.

Relation Between Exercise Variables and Delta-LVDd

To examine a possibility that excessive exercise intensity or frequency might be associated with LV remodeling, correlations between exercise variables (ie, prescribed training heart rate, frequency of participation in exercise training sessions, and the increase and percentage increase in peak $\dot{V}O_2$ at the end of the 12-week program) and delta-LVDd were assessed (Table 4). The ranges of distribution of these variables were 75–140 beats/min for prescribed training heart rate, 2–76 attendances at exercise sessions, –348–692 ml/min for the increase in peak $\dot{V}O_2$, and –32–64% for percentage increase in peak $\dot{V}O_2$ for all patients. These variables were considered to reflect the intensity, frequency and overall amount of exercise training that could potentially affect the development of LV remodeling. However, none of these variables significantly correlated with delta-LVDd (Table 4), suggesting that exercise training with appropriate intensity and frequency is not associated with LV remodeling.

Multivariate Analysis

Multiple linear regression analysis using 2 variables (the baseline plasma BNP concentration and infarct location, which affected the development of LV remodeling in the univariate analysis) indicated that only the baseline plasma BNP concentration significantly affected the development of LV remodeling ($p=0.046$), whereas the infarct location (anterior) had a tendency to affect the development of LV remodeling ($p=0.18$).

The combination of the baseline plasma BNP concentration and infarct location (anterior) improved the specificity and positive predictive value for delta-LVDd >5 mm better than the individual variables (Table 5).

Table 4 Effect of Exercise Intensity and Frequency on Delta-LVDd

	r	*p value*
Training heart rate	*0.07*	*NS*
Frequency of participation in the rehabilitation program	*–0.19*	*NS*
Delta peak $\dot{V}O_2$	*–0.01*	*NS*
% Delta peak $\dot{V}O_2$	*0.11*	*NS*

Discussion

The major findings of the present study of patients with AMI participating in a 12-week exercise CR program are that (1) delta-LVDd at 1 year after the end of the program was significantly greater in patients with an anterior MI than with other infarct locations ($p<0.05$), (2) delta-LVDd correlated significantly with the baseline BNP concentration ($p<0.05$) and delta-LVDd >5 mm occurred exclusively in patients with a baseline BNP concentration higher than 150 pg/ml, and (3) variables representing the intensity and frequency of exercise training did not significantly correlate with delta-LVDd. These findings suggest that in patients with AMI participating in exercise CR, the baseline plasma BNP concentration and infarct location, but not exercise intensity or frequency, are factors influencing the development of LV remodeling.

Previous Studies

The EAMI study[9] and Dubach et al[23] have demonstrated that exercise training does not aggravate LV remodeling, and the ELVD study[10] has shown that exercise training instead attenuates LV remodeling. However, these studies did not analyze the predictive factors of LV remodeling in

Table 5 Sensitivity, Specificity and Positive (PPV) and Negative (NPV) Predictive Values of Baseline Plasma BNP Concentration and the Infarct Location (anterior) Against Delta-LVDd >5 mm

	Sensitivity (%)	*Specificity (%)*	*PPV (%)*	*NPV (%)*
Anterior infarction and BNP >150 pg/ml	*75.0*	*68.8*	*23.1*	*95.6*
Anterior infarction	*75.0*	*53.1*	*16.7*	*94.4*
BNP >150 pg/ml	*100*	*45.3*	*18.6*	*100*

the patients participating in exercise training. In addition, in those studies, exercise training was started relatively late (3–8 weeks) after the onset of infarction, which is not recent trend of early discharge and early return to work after AMI[24] Thus, factors predicting subsequent LV remodeling in patients participating in exercise CR, starting early (approximately 2 weeks) after the onset of the AMI remain unknown. Recently, we demonstrated that patients with a low LVEF do not have aggravated LV remodeling after moderate intensity exercise training starting 2 weeks after the onset of AMI[25] However, neurohumoral or exercise variables were not analyzed in that study.

Present Study

Compared with previous studies, the present study is unique because it comprehensively analyzed the predictive factors of LV remodeling using clinical, angiographic, neurohumoral, and exercise variables at baseline in postinfarction patients participating in exercise CR. As far as we know, no previous study has performed such a comprehensive analysis to identify determinants of postinfarction LV remodeling.

In addition, the present study is unique because our exercise rehabilitation program starts relatively early (approximately 2 weeks) after the onset of AMI. Although this timing may not be very early when compared with the recent trend of early discharge (3–5 days) after AMI[24] it is much earlier than the timing (3–8 weeks after the onset) in the previous EAMI and the ELVD studies.

Plasma BNP Concentration and Ventricular Remodeling

The present findings that anterior infarct location and baseline plasma BNP concentration are major influencing factors of LV remodeling are in accordance with previous reports[12–14] We have shown in our previous studies that an initial elevation (>100 pg/ml on the 7th day after onset) and a subsequent sustained elevation (percentage decrease <25% from 30th to 90th day) of plasma BNP are reliable predictors of progressive LV remodeling after acute MI[13,14] The present result indicates that our previous findings also hold true in patients participating in exercise CR after AMI. In addition, the present results suggest that patients with both anterior infarct location and an elevated BNP concentration (>500 pg/ml) at baseline are at high risk of subsequent LV remodeling.

Hama et al reported that the expression of the rat ventricular BNP gene after MI mainly occurred at the border of the infarcted region, where mechanical wall stress may be maximal[26,27] Cerisano et al reported that LV dilatation occurring early after infarction is predictable from the Doppler-derived mitral deceleration time indicating an elevated LV filling pressure[28] These findings suggest that a high plasma BNP concentration may reflect elevated LV wall stress, which is likely to accelerate LV remodeling and may explain why a high plasma BNP concentration is predictive of LV remodeling. Although the determination coefficient (r^2=0.09) of plasma BNP concentration for LV remodeling was not remarkably high in the present study, the results suggest that plasma BNP concentration is one of many determinants of the complex process of LV remodeling.

Exercise Intensity and Ventricular Remodeling

The present study found that exercise variables representing intensity, frequency, and total amount of exercise in CR do not affect LV remodeling, even when the exercise training is started relatively early (approximately 2 weeks) after the onset of AMI. The reasons for this result may be two-fold. First, the impact of the plasma BNP concentration is so powerful that the influence of exercise intensity or frequency on ventricular remodeling is masked. In other words, a transient increase in LV wall stress by exercise training may have no or only a small impact on the development of LV remodeling compared with the impact of baseline wall stress determined by infarct size. This possibility is supported by the result of the EAMI study showing that LV remodeling developed in patients with low LVEF regardless of exercise training[9]

A second potential explanation is that the prescribed exercise intensity (40–60% of heart rate reserve) and frequency were at appropriate levels for not aggravating LV remodeling. In fact, a slightly lower exercise intensity (40–50% of heart rate reserve) was prescribed for patients with low LVEF (<40%) in the present study. This suggests that exercise intensity or frequency is not associated with LV remodeling, as long as they are within an appropriate range in the CR program. If more vigorous and excessive exercise had been imposed, LV remodeling might have occurred, a possibility that is supported by previous experimental results showing that vigorous swimming exercise in rats after a large MI aggravated LV remodeling[29,30] whereas a moderate level of exercise either did not adversely affect[31] or even favorably attenuated LV remodeling in the same rat model[32]

Clinical Implications

On the basis of the present results, plasma BNP concentration and infarct location are useful tools for predicting the likelihood of LV remodeling before beginning an exercise CR program after AMI. The present results also suggest that a moderate exercise intensity (50–60% heart rate reserve) for patients with LVEF >40% and a slightly lower intensity (40–50% heart rate reserve) for those with LVEF <40% may be safe and appropriate for average patients participating in exercise CR after AMI. Because patients with an anterior infarction and BNP concentration >150 pg/ml at approximately 2 weeks after the onset (especially, >500 pg/ml) are at high risk for subsequent LV remodeling and these patients often overlap with those with LVEF <40%, a relatively low level of exercise intensity (40–50% of heart rate reserve) is recommended for these patients. A low to moderate level (50% of peak $\dot{V}O_2$) of exercise has been reported to increase exercise capacity while minimizing ventricular wall stress in patients with LV dysfunction[33] To determine whether a higher level of exercise intensity (50–70% heart rate reserve) aggravates LV remodeling, further studies are needed.

Study Limitations

The present study was a prospective observational study, and did not have a sedentary control group. Although this might have affected the association between exercise intensity and LV remodeling, the wide variation of exercise intensity and frequency does allow us to analyze correlations between exercise variables and delta-LVDd.

A one-dimensional measure, such as delta-LVDd, has a certain limitation in the assessment of 3-dimensional LV remodeling. We intentionally avoided LV end-systolic dimension (LVDs) as an index of LV remodeling, because LVDs suffers from a greater error than LVDd when local

asynergy of LV wall motion exists[25]

The presence of myocardial ischemia may have affected both delta-LVDd and peak $\dot{V}O_2$; however, we excluded patients presenting with myocardial ischemia in the initial exercise test.

Conclusion

In patients with AMI participating in exercise CR, baseline plasma BNP concentration and anterior infarct location, but not exercise intensity or frequency, are predictive factors of the development of LV remodeling. Patients with both anterior infarction and a BNP concentration >150 pg/ml at approximately 2 weeks after onset are at high risk for subsequent LV remodeling.

Acknowledgment

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Circ J 2004; **68:** 304–307

Exercise-Induced Hepatocyte Growth Factor Production in Patients After Acute Myocardial Infarction

—— Its Relationship to Exercise Capacity and Brain Natriuretic Peptide Levels ——

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Background The hepatocyte growth factor (HGF) is a multifunctional cytokine with cardioprotective properties and potent myogenic activity for vascular endothelium. In patients after acute myocardial infarction, exercise training has the beneficial effects on cardiovascular adaptations. We hypothesized that exercise induces HGF production in those patients. If this hypothesis is correct, HGF production may be associated with clinical parameters of cardiovascular function.

Methods and Results In 20 patients after acute myocardial infarction, HGF levels in the pulmonary artery (HGF_{PA}) and aorta (HGF_{Ao}) were determined at rest and during supine submaximal exercise, with cardiac output (CO) measured by catheterization. Exercise-induced HGF production was calculated by using the following equation: [($HGF_{PA}-HGF_{Ao}$)×CO during exercise]−[($HGF_{PA}-HGF_{Ao}$)×CO at rest]. On a separate day, peak oxygen uptake ($\dot{V}O_2$) was determined during a symptom-limited upright cardiopulmonary exercise test. Exercise increased HGF production (from 1.6±3.0 to 9.0±6.3 μg/ml, $p<0.001$). Exercise-induced HGF production was inversely related to peak $\dot{V}O_2$ ($r=-0.664$, $p<0.01$) and positively related to levels of brain natriuretic peptide (BNP), a biochemical marker for post-infarction ventricular remodeling ($r=0.686$, $p<0.01$).

Conclusions Exercise significantly increases HGF production. This phenomenon may play an important role in post-infarction patients, particularly with reduced exercise tolerance and elevated BNP levels. (*Circ J* 2004; **68:** 304–307)

Key Words: Exercise; Growth substances; Myocardial infarction; Rehabilitation

Hepatocyte growth factor (HGF), originally identified and cloned as a potent mitogen for hepatocyes, has mitogenic, motogenic, morphogenic, and anti-apoptotic activities in a variety of cells through its receptor, c-Met.[1,2] HGF is a unique growth factor to act protectively against endothelial dysfunction[3–5] myocardial ischemia/infarction and remodeling.[6–8] Thus, the HGF system (HGF and its receptor c-Met) is attracting increasing attention in the field of cardiovascular pathophysiology.[9]

In patients with acute myocardial infarction (AMI), exercise training has the beneficial effects on cardiovascular systems.[10] Cardiac effects include attenuation of post-infarction ventricular remodeling,[11,12] for which brain natriuretic peptide (BNP) is a useful biochemical marker.[13] Vascular effects include an increase in the density of skeletal-muscle capillaries[14] and improvement in endothelial-dependent vasodilation,[15,16] which are important determinants for exercise tolerance and symptoms.

In the present study, we hypothesized that exercise induces HGF production, mediating the beneficial effects of exercise training in patients with AMI. If so, HGF production may be associated with clinical parameters of cardiovascular function.

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Methods

Study Patients

The study group included 20 male patients (aged 61± 12 years [mean±SD]) after AMI. The infarction site was anterior in 13 patients (65%), and inferior/lateral in 7 patients (35%). All patients underwent reperfusion therapy (percutaneous transluminal coronary angioplasty in 17 patients and intravenous administration of tissue-type plasminogen activator in 3 patients) on admission. The peak level of serum creatine kinase was 2,995±2,043 [mean± SD] U/L. The severity of heart failure ranged from New York Heart Association functional class I to II. The baseline patient characteristics are summarized in Table 1. No patients had liver (elevated levels of aminotransferases), kidney (elevated levels of creatinine or urea), or lung dysfunction (restrictive or obstructive pattern in spirometry). No patients had prior myocardial infarction. Medications remained unchanged during the entire study.

The study was approved by the institutional review committee. The protocol was fully explained, and all patients gave their written informed consent to participate in the study.

Table 1 Baseline Characteristics of Patients

NYHA, n (%)	
I	*12 (60)*
II	*8 (40)*
LVEF (%)	*43±9*
LVEDVI (ml/m²)	*70±11*
LVEDP (mmHg)	*13±6*
Coronary risk factors, n (%)	
Diabetes mellitus	*11 (55)*
Hyperlipidemia	*9 (45)*
Hypertension	*7 (35)*
Medications, n (%)	
ACE-inhibitor	*11 (55)*
Ca^{2+}-antagonist	*12 (60)*
Nitrates	*9 (45)*
Aspirin	*20 (100)*
Diuretics	*8 (40)*
Digoxin	*7 (35)*
β-blockers	*4 (20)*

NYHA, New York Heart Association classification; LVEF, left ventricular ejection fraction; LVEDVI, left ventricular end-diastolic volume index; LVEDP, left ventricular end-diastolic pressure; ACE, angiotensin-converting enzyme; Ca^{2+}, calcium.

Table 2 Changes in Hemodynamics and HGF Levels in Response to Supine Exercise

	Baseline	*Peak exercise*	*p value*
HR (beats/min)	*68±11*	*117±17*	*<0.001*
Aosyst (mmHg)	*126±17*	*169±20*	*<0.001*
PAsyst (mmHg)	*34±7*	*58±14*	*<0.001*
PAdiast (mmHg)	*11±4*	*19±5*	*<0.001*
CO (L/min)	*6.8±1.6*	*14.7±3.9*	*<0.001*
HGF_{PA} (ng/ml)	*7.72±3.50*	*7.82±3.53*	*NS*
HGF_{Ao} (ng/ml)	*7.45±3.32*	*7.14±3.14*	*NS*
ΔHGF(PA–Ao) (ng/ml)	*0.27±0.44*	*0.68±0.58*	*<0.01*
CO×ΔHGF (μg/min)	*1.6±3.0*	*9.0±6.3*	*<0.001*
$S\dot{V}O_2$ (%)	*67±4*	*34±10*	*<0.001*

HGF, hepatocyte growth factor; HR, heart rate; Ao, aorta; PA, pulmonary artery; syst, systolic pressure; diast, diastolic pressure; CO, cardiac output (by the thermodilutional method); ΔHGF (PA–Ao), the difference in HGF levels between pulmonary artery and aorta; $S\dot{V}O_2$, mixed venous O_2 saturation.
p values were assessed with the paired student t-test.

Cardiac Catheterization and Supine Exercise Test

From the right brachial artery through a 6F sheath, chronic phase coronary angiography and left ventriculography were performed according to the conventional Judkins technique, 28±7 [mean±SD] days after the onset of myocardial infarction[17] Heparin was initially administered at a dose of 5,000 IU into the distal brachial artery. For angiographic evaluation of left ventricular volumes, ventricular silhouettes in 30° right anterior oblique projections were digitized with an ANCHOR ventriculography analysis system (Siemens-Elema, Solna, Sweden). By the area-length method, the left ventricular end-systolic and end-diastolic volume indices and ejection fraction were calculated. Left ventricular pressure was measured with a 2F high-fidelity micromanometer catheter (model SPC-320; Miller Instruments, Houston, TX, USA) advanced into the left ventricle via the lumen of a 6F pig tail catheter.

A 7.5 F Swan-Ganz thermodilution catheter (Opticath®; Abbott Laboratories, North Chicago, IL, USA) was inserted through the left subclavian vein, to measure cardiac output (CO) and pulmonary artery (PA) pressure.

After sampling blood and measuring hemodynamic parameters at baseline, the supine bicycle exercise test was performed by using a Siemens Ergometry System 930B, and the mixed venous O_2 saturation ($S\dot{V}O_2$), and pressure of the PA and aorta (Ao) were monitored. We also monitored arterial blood O_2 saturation continuously using a Biox III pulse oximeter (Omeda, Louisville, KY, USA). The workload was increased at 3-min intervals in 30-W increments followed by a 0-W bicycling period for 1 min. The exercise was finished at 30 W in 2 patients, 60 W in 8 patients and 90 W in 10 patients. This final workload was the submaximal level for each patient, because the peak heart rate was approximately 80% of the maximal heart rate achieved at the symptom-limited cardiopulmonary exercise test, as described below. Before and immediately after the supine exercise test, blood samples were taken from the PA and the Ao. The samples were centrifuged at 4°C and stored at −80°C until assayed.

Cardiopulmonary Exercise Test

On a separate day (3±1 [mean±SD] days before the cardiac catheterization), patients underwent the symptom-limited cardiopulmonary exercise test (CPX), with determination of peak oxygen uptake ($\dot{V}O_2$), workload and heart rate. The exercise test was performed on a calibrated, electronically braked bicycle in an upright position (Examiner, Lode B.V., Groningen, Netherlands). Ramp protocols began at a workload of 0 W for 1 min and increased in 15-W increments at 1-min intervals. Expired gas analysis was performed by using a respiromonitor AE-280 (Minato Products, Tokyo, Japan). The $\dot{V}O_2$ was measured on a breath-by-breath basis, and was averaged over contiguous 30-s intervals, except at peak exercise, when 18-s averaging was used.

Hepatocyte Growth Factor Measurements

Hepatocyte growth factor levels in the pulmonary artery (HGF_{PA}) and aorta (HGF_{Ao}) were determined with specific enzyme-linked immunosorbent assay kits (Otsuka Assay Laboratories, Tokushima, Japan). Microtiter plates coated with an anti-HGF murine monoclonal antibody were incubated with standard HGF or serum samples, and an anti-HGF rabbit polyclonal antibody was added. After adding first the anti-rabbit goat immunoglobulin G-peroxidase conjugate and then o-phenylene diamine, the absorbance was read at 492 nm using a plate reader[18] The sensitivity of the HGF kit was 0.1 ng/ml. This assay system detects only bioactive, heterodimeric (mature) forms of HGF in the blood samples[18,19] Previous studies have demonstrated that there is a strong (r=0.986) positive correlation between HGF levels measured by this assay system and those measured by bioassay (determined by stimulating DNA synthesis of rat hepatocytes in primary cultures)[18,19]

The BNP levels were determined with a specific immunoradiometirc assay kit (Shionogi Co, Osaka, Japan), as previously reported[17] The sensitivity of this BNP kit is 2 pg/ml. Brain natriuretic peptide has been considered as a biochemical marker of ventricular remodeling after myocardial infarction.

Data Analysis

Exercise-induced HGF production (μg/min) was calculated by using the following equation:

$[(HGF_{PA}-HGF_{Ao})\times CO$ at peak exercise$]-[(HGF_{PA}-HGF_{Ao})\times CO$ at rest$]$.

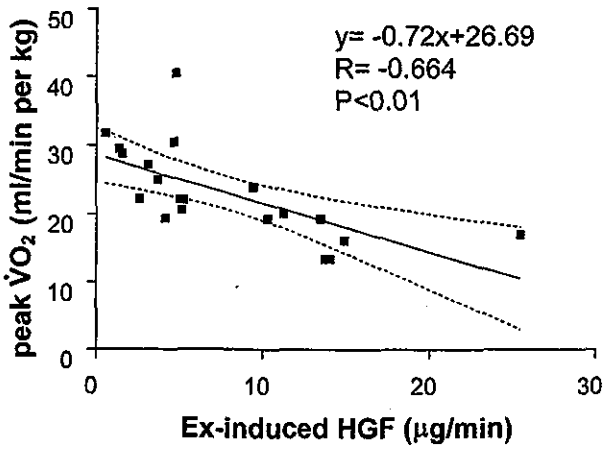

Fig 1. Correlation of the exercise (Ex)-induced hepatocyte growth factor (HGF) production with peak oxygen uptake (peak $\dot{V}O_2$) in 20 patients after acute myocardial infarction (AMI).

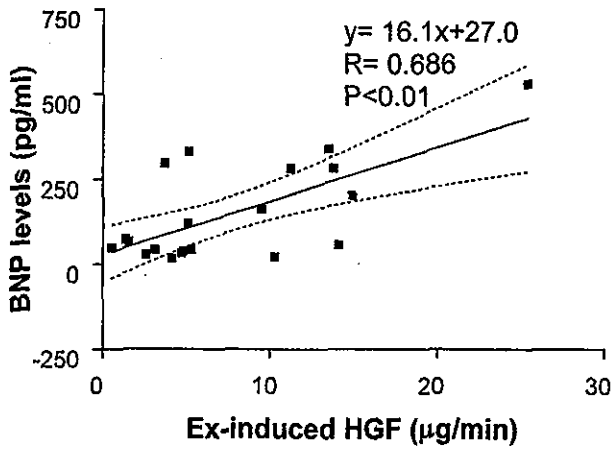

Fig 2. Correlation of the exercise Ex-induced HGF production with brain natriuretic peptide (BNP) levels.

The χ^2 test was used for comparison of categorized variables. The Student's t-test or Mann-Whitney U-test rank test was used for comparisons of mean values to determine significance of difference between the 2 groups. Linear regression curves and correlations were calculated according to the least squares method. All data are presented as mean±SD. Differences were considered significant at $p<0.05$.

Results

Changes in Hemodynamics and HGF in Response to Supine Exercise

Table 2 shows the changes in hemodynamics and HGF levels, at baseline (=before exercise) and at peak exercise during the catheterization. At baseline, there were no significant differences in HGF levels between PA and Ao. The supine exercise (74±18 W in intensity, 10±2 min in duration) significantly increased heart rate, Ao pressure, PA pressure, and CO, whereas it decreased $S\dot{V}O_2$. Although the absolute HGF levels in PA and Ao appear unchanged, the difference in HGF levels between PA and Ao (ΔHGF) significantly increased by approximately 3-fold after the exercise. When assessed based on the fold change compared with the baseline level, exercise increased the HGF_{PA} to 1.02±0.11-fold ($p<0.05$), but did not change HGF_{Ao} (0.96±0.09-fold). Finally, in the patients of the present study, exercise-induced HGF production ([ΔHGF×CO at peak exercise]−[ΔHGF×CO at baseline]) was calculated to be 7.4±6.3 μg/min, on average.

Correlations With HGF Production

Peak $\dot{V}O_2$, workload and heart rate determined during the symptom-limited upright cardiopulmonary exercise text (CPX) performed on a separate day were 23±7 ml/min per kg, 130±37 W, and 140±24 beats/min, respectively.

As shown in Fig 1, exercise-induced HGF production correlated inversely with peak $\dot{V}O_2$ ($r=-0.664$, $p<0.01$). Eight patients with peak $\dot{V}O_2<20$ ml/min per kg had greater exercise-induced HGF production (13.4±5.9 vs 3.9±2.4 μg/min, $p<0.05$) and higher prevalence of angiographically significant stenosis in major coronary arteries (>60%) (50 vs 8%, $p<0.05$) in comparison with the remaining 12 patients with $\dot{V}O_2\geq20$ ml/min per kg.

Also, as shown in Fig 2, exercise-induced HGF production correlated positively with BNP levels at baseline ($r=0.686$, $p<0.01$). However, there were no significant relations with cardiac function at rest (left ventricular end-diastolic volume index and ejection fraction), percentage increase in heart rate, Ao pressure and PA pressure in response to the submaximal supine exercise (data not shown).

Discussion

The major finding of the present study is that HGF production is induced during exercise in accordance with the severity of exercise intolerance and the increase in BNP levels.

In patients after AMI, exercise training is now emerging as an important component of the therapy[10,20] It attenuates post-infarction ventricular remodeling, which is associated with heart failure and increased mortality[21] and is accompanied by an elevated level of BNP[13] Regular exercise training also increases the density of skeletal muscle capillaries[14] and induces repetitive increases in vascular blood flow and shear stress[22] thereby improving endothelium-dependent vasodilation[15,16] Both central (cardiac) and peripheral (skeletal muscle and vascular) effects of exercise training may consequently improve exercise tolerance and symptoms[10] From the data obtained in the present study, a causal relationship cannot be clearly determined. However, the several effects of exercise training are potentially mediated through HGF in patients after AMI.

As shown in Table 2, exercise increases the concentration gradients of HGF levels between PA and Ao, indicating exercise-induced HGF production. The vessel wall may be a potential source of circulatory HGF[23] Fig 1 shows that exercise-induced HGF production is associated with reduced peak $\dot{V}O_2$. In particular, patients with peak $\dot{V}O_2<20$ ml/min per kg were sensitive towards the HGF response to exercise. These patients with reduced exercise capacity had a higher prevalence of coronary artery stenosis. Myocardial ischemia appears to be one of the determinants for exercise capacity and is known to induce upregulation of non-cardiac HGF systems[24] HGF promotes angiogenesis as a potent growth factor of endothelial cells[25] and promotes the functional recovery of nitric-oxide-mediated vasodila-

tion?[26] thus improving myocardial blood flow. Also, as shown in Fig 2, exercise-induced HGF production is associated with increased levels of BNP. The HGF may be systemically released during exercise in response to left ventricular dysfunction and may exert wound healing and cardioprotective actions against myocardial ischemia/infarction?[27,28] In a mouse myocardial infarction model, HGF gene therapy attenuated left ventricular remodeling and dysfunction?[28]

The recent clinical studies also suggest a possibility that HGF may contribute to improving myocardial ischemia and dysfunction. In the CAPTURE (c7E3 Anti-Platelet Therapy in Unstable REfractory angina) trial studying the patients with acute coronary syndromes, elevated HGF levels are associated with reduced incidence of death and myocardial infarction?[29] In another study in patients with coronary artery disease, elevated coronary sinus HGF levels were associated with collateral formation and with left ventricular dysfunction?[30] Thus, interventions that enhance HGF levels could be beneficial in the management of those patients. Exercise is a potential approach. However, further studies are required to determine whether short-term benefits of exercise could translate into long-term effects?[20]

In conclusion, the present study provides a novel aspect of exercise training as cytokine-mobilization. HGF may have a therapeutic implication in patients after AMI.

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SCIENTIFIC LETTER

p53Arg72Pro polymorphism of tumour suppressor protein is associated with luminal narrowing after coronary stent placement

S Kojima, N Iwai, N Tago, K Ono, K Ohmi, G Tsujimoto, S Takagi, S Miyazaki, H Nonogi, Y Goto

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The cause of in-stent luminal narrowing has been primarily considered to be neointimal hyperplasia that is caused by proliferating vascular smooth muscle cells (VSMC). It has been recently reported that local drug delivery systems produce good results for the inhibition of VSMC proliferation.[1] The potential of suppressive agents in the treatment of in-stent luminal narrowing arises from basic studies according to cell cycle regulation and gene expression.[1]

p53 is a tumour suppressor protein involved in regulating the growth of VSMC. Loss of p53 activity results in the growth of VSMC, while increased concentrations of p53 result in apoptosis of VSMC. A common polymorphism in the p53 amino acid sequence which results in the presence of either arginine (Arg) or proline (Pro) at position 72 may influence the susceptibility to malignancy through its interaction with p73. The effects of this polymorphism on p53 function seem to be related to p73, and p53Arg was reported to be more susceptible to the inactivation of p73 than p53Pro alleles.[2]

It is conceivable that this common polymorphism of p53, Arg72Pro, may also influence VSMC proliferation after coronary stent implantation. In the present study, we tested this hypothesis in patients after coronary stent implantation using quantitative coronary angiography.

METHODS

The study population was selected from outpatients at the National Cardiovascular Center, Osaka, Japan, who underwent follow up coronary angiography after successful stent placement. This genetic study was approved by our institutional ethics committee and included 132 consecutive patients admitted between August and October 1999, from whom informed consent was obtained. Major adverse cardiac events (death, myocardial infarction, coronary artery bypass graft surgery, and repeat interventions) did not occur in any of the patients undergoing stent implantation, as assessed by follow up angiography.

Quantitative computer assisted angiographic measurements were performed on end diastolic frames using an automated edge detection system CMS (MEDIS Medical Imaging Systems, Leiden, The Netherlands). The minimal lumen diameter (MLD), reference lumen diameter (RLD), and per cent diameter stenosis were obtained using this system.

Genomic DNA was extracted from peripheral blood leucocytes. The p53 genotype was determined using the TaqMan system, which combines polymerase chain reaction amplification and detection in a single closed tube. The primers and probes were used for allelic discrimination of p53Arg72Pro polymorphism. The validity of the TaqMan system was confirmed using DNA samples of the three genotypes as confirmed by direct sequencing.

VSMC were prepared from the aorta of p53 knockout mice. Transient transfection of the p53 gene was performed using Lipofect Amin (Gibco BRL) according to the instructions of the manufacturer. VSMC were plated onto a 96 well multititre plate (10 000 cells/well) and cultured in Dulbecco's modified Eagle's medium (DMEM) and 10% fetal bovine serum (FBS) for 24 hours. After deprivation of serum for 24 hours, VSMC were transfected with wild/mutant p53 plasmids (n = 48). Transfected VSMC were cultured in DMEM and 2% FBS for 48 hours. Forty eight hours after the transfection, the cell number was counted with a WST (water soluble tetrazolium salt) cell counting kit (Wako). The expression construct of p53 was purchased from Invitrogen (GeneStorm expression-ready human clones). p53 cDNA is expressed under the control of a cytomegalovirus promoter. p53Pro was made from p53Arg by in vitro mutagenesis. The sequences of both constructs were confirmed by direct sequencing.

RESULTS

The 132 stenting lesions were divided into three groups according to the p53 genotype (47 Arg/Arg, 64 Arg/Pro, and 21 Pro/Pro). These genotype frequencies were compatible with the Hardy-Weinberg equilibrium. There were no significant differences in RLD among the three genotypes (3.00 (0.39) mm *v* 3.00 (0.44) mm *v* 3.05 (0.35) mm). MLD did not significantly differ before (0.46 (0.47) mm *v* 0.52 (0.51) mm *v* 0.48 (0.48) mm) or immediately after stent implantation (2.82 (0.47) mm *v* 2.74 (0.58) mm *v* 2.79 (0.42) mm) (fig 1A). However, MLD was significantly smaller (1.36 (0.86) mm *v* 1.83 (0.90) mm *v* 2.01 (0.92) mm, $p < 0.005$) and the per cent diameter stenosis was significantly greater (55 (27)% *v* 41 (26)% *v* 34 (25)%, $p < 0.005$) in the Arg/Arg genotype than in the other groups at follow up (fig 1B).

To assess the determinants of MLD at follow up, a multiple regression analysis with a backward elimination was performed. The results revealed that MLD at follow up was determined ($r = 0.531$, $p < 0.0001$) by the p53 genotype ($p = 0.001$), RLD ($p < 0.005$), diabetes mellitus ($p < 0.05$), and MLD immediately after stent implantation ($p < 0.05$).

To confirm the effect of the p53 genotype on VSMC we transfected Arg and Pro of p53 genes to aortic VSMC from p53 knockout mice. The cell count after transfection with p53Arg (25 423 (4022), n = 48) was greater than that after transfection with p53Pro (18 623 (2538), n = 48) ($p < 0.0001$).

Abbreviations: Arg, arginine; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; MLD, minimal lumen diameter; Pro, proline; RLD, reference lumen diameter; VSMC, vascular smooth muscle cells

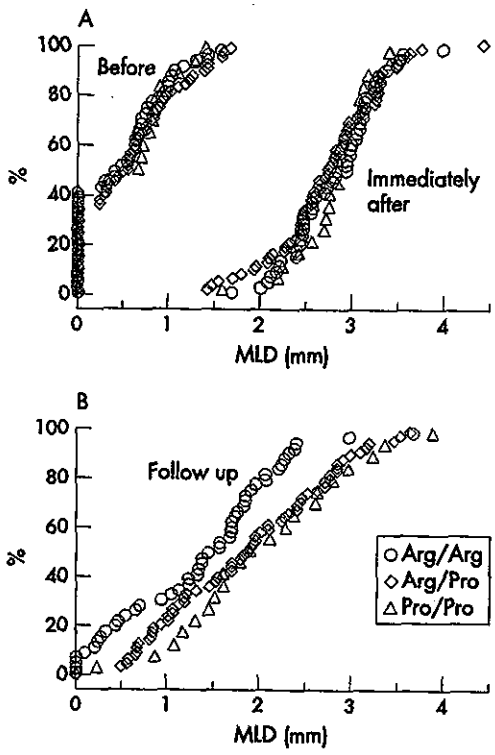

Figure 1 (A) Cumulative distribution curves of the MLD before and immediately after stent implantation for each genotype of p53. (B) Cumulative distribution curves of MLD at the time of follow up after stent implantation for each genotype of p53.

DISCUSSION

In the present study, we found that MLD at follow up angiography was significantly smaller in the Arg/Arg genotype than in the Arg/Pro and Pro/Pro genotypes of p53 in patients who underwent coronary stenting, and that MLD at follow up was determined by the p53 genotype, RLD, diabetes mellitus, and MLD immediately after stent implantation.

p53 has been shown to induce cell cycle arrest at the G_1/S boundary and also apoptosis. Increased concentrations of p53 result in apoptosis of VSMC, while loss of p53 activity results in the growth of VSMC. Guevara and co-workers reported the excessive proliferation of VSMC at S phase as a result of p53 inactivation in vivo.[3] Taken together, the present results suggest that p53Arg72Pro polymorphism is related to the functional activity of p53, which regulates VSMC proliferation leading to luminal narrowing after stent placement.

We also demonstrated that p53Arg has less of an inhibitory effect on VSMC proliferation than p53Pro. The functional difference between p53 variants was observed in a recent study which showed that the p53Arg variant is more efficient than p53Pro at inducing apoptosis, and that one mechanism underlying this greater efficiency is enhanced localisation of the p53Arg variant to mitochondria.[4] However, the localisation of p53 to mitochondria seems to occur only in tumour cells.[5] Therefore, this p53Arg induced apoptosis is unlikely to be the cause of our findings. On the other hand, the manner of p53 according to the codon 72 polymorphic variants may be deeply affected by p73. Marin and colleagues[2] recently reported that the Arg/Arg genotype in squamous cell tumours may reduce the inhibition of cell growth, possibly because the conformational p53 protein, consisting of the homozygote for p53Arg, can bind to the p73 protein, and neutralise p73-induced apoptosis. This indicates that interaction of the p53 protein with the p73 protein is influenced by a common polymorphism of p53 at amino acid residue 72. Consequently, VSMC proliferation may occur because p53Arg tends to block p73 function more effectively than p53Pro.

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Local Delivery of Argatroban for the Prevention of Restenosis After Coronary Balloon Angioplasty

— A Prospective Randomized Pilot Study —

Tomonori Itoh, MD; Hiroshi Nonogi, MD; Shunichi Miyazaki, MD; Akira Itoh, MD; Satoshi Daikoku, MD; Isao Morii, MD; Yoichi Goto, MD for the 3D-CAT investigators

Background Effective pharmacological prevention of restenosis using the systemic administration of various drugs that were effective for the prevention of restenosis in experimental studies has not been reported. The purpose of this study was to evaluate whether the local delivery of a potent thrombin inhibitor, argatroban, using a local drug delivery device would prevent restenosis after plain old balloon angioplasty (POBA).

Methods and Results Seventy patients with chronic coronary artery disease requiring POBA were randomly assigned to wither the control group (n=35) or the argatroban group (n=35). In the argatroban group, argatroban was administered intravenously for 30 min before the POBA and intracoronarily into the dilated site using a Dispatch™ catheter immediately after the POBA, followed by a postoperative intravenous infusion for 4 h. The angiographical lesion restenosis and clinical restenosis rates at follow-up were significantly lower in the argatroban group (27% and 14%) than in the control group (56% and 37%; p=0.02 and p=0.03, respectively). There was no major complication during the procedure.

Conclusion The local delivery of argatroban is safe and effective in preventing restenosis after balloon angioplasty. (*Circ J* 2004; **68:** 615–622)

Key Words: Coronary angioplasty; Direct thrombin inhibitor; Local drug delivery; Restenosis

The clinical efficacy of coronary balloon angioplasty (plain old balloon angioplasty: POBA) is limited by restenosis, which occurs in 30–50% of cases despite a successful procedure[1–4] However, in previous clinical trials[5–9] there has not been effective pharmacological prevention of restenosis using the systemic administration of various drugs that were found to be effective for the prevention of restenosis in experimental studies. One of the major factors in the failure of restenosis prevention in these clinical trials could be that the systemic administration of drugs resulted in a concentration at the site of a balloon injury that was too low. Accordingly, it has been anticipated that the local delivery of a drug at a high concentration may reduce the restenosis rate after POBA. However, the pharmacological prevention of restenosis using a local drug delivery system has not yet been tested in clinical trials except for one small-scale trial[10]

It was recently reported that the messenger RNA (mRNA) of a thrombin receptor is expressed in medial smooth muscle cells in the very early phase after a balloon catheter injury (within 6 h)[11,12] Moreover, pre-treatment with hirudin (a direct thrombin inhibitor) was found to reduce vascular lesion development after balloon injury in experimental studies[13,14] Thus, it is thought that restenosis may be prevented or minimized by the local administration of a direct thrombin inhibitor. Argatroban is a direct thrombin inhibitor that has a more potent inhibitory effect on fibrin- or clot-incorporated thrombin than other thrombin inhibitors such as heparin and hirudin[15,16] Tomaru *et al* reported that the local delivery of argatroban using a double-balloon catheter reduced intimal thickening after balloon injury in an experimental study[17] Accordingly, we conducted a prospective, randomized, controlled clinical trial to assess the effect of the local delivery of argatroban as a direct thrombin inhibitor using a Dispatch™ catheter system[18] (SIMED Life Systems, Inc, Maple Grove, MN, USA) in the prevention of restenosis after percutaneous coronary intervention (PCI).

Methods

Study Protocol

Between March 1995 and May 1997, 70 patients who required coronary revascularization were registered in the present trial (Drug Delivery Device in Coronary Balloon Angioplasty Trial: 3D-CAT) at the National Cardiovascular Center. The 3D-CAT is a randomized controlled pilot trial for prevention of restenosis after coronary balloon angioplasty conducted at a single center. The inclusion criterion was that the patient was scheduled for elective POBA with a balloon size equal to or larger than 2.75 mm. All of the patients had ischemic chest pain or evidence of ischemia diagnosed by a thallium-201 or treadmill exercise test. The patients were randomly assigned to 2 groups

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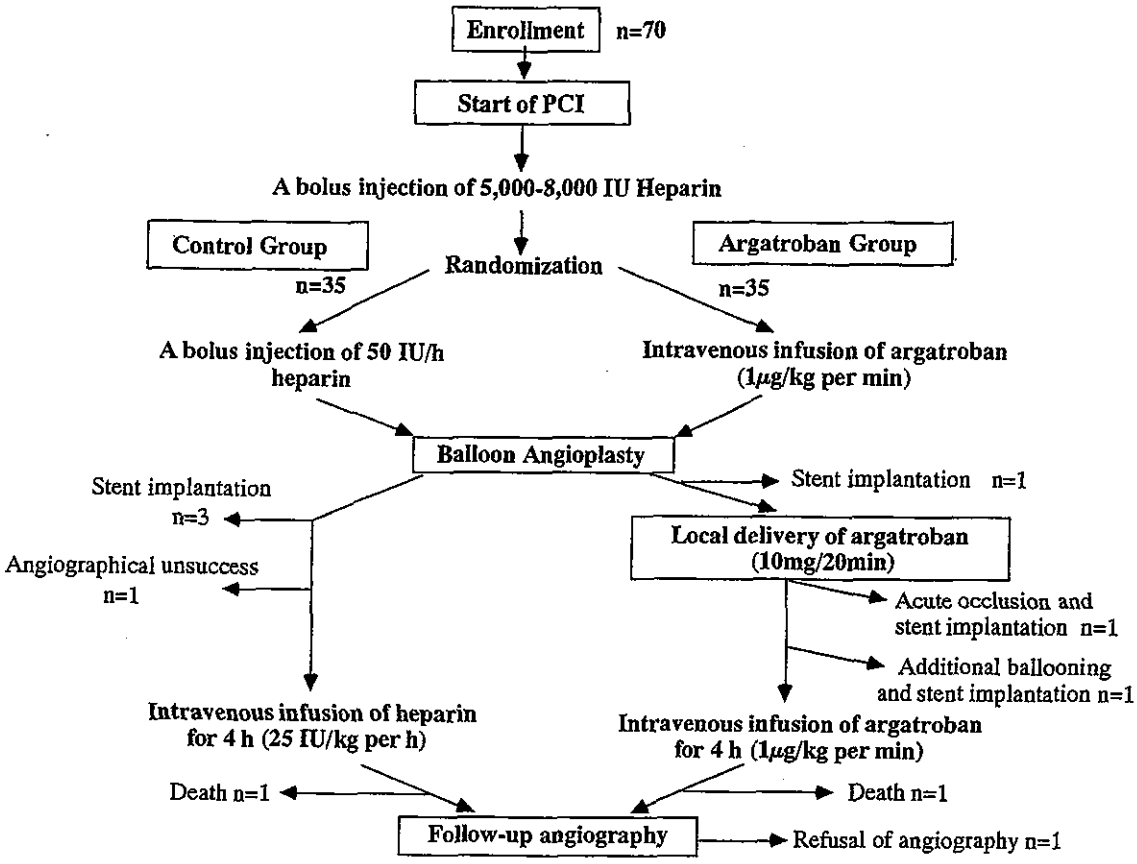

Fig 1. Study protocol and patient flow chart. A bolus of 5,000–8,000 IU heparin was injected intravenously at the start of the percutaneous coronary intervention (PCI) procedure. Patients were randomly assigned to 2 groups: the control group and the argatroban group receiving local delivery of argatroban via a Dispatch™ catheter after PCI. Control group: PCI was performed; the patients received a bolus injection of heparin during the procedure and an intravenous infusion of heparin for 4 h after angioplasty. Argatroban group: intravenous infusion of argatroban was started 30 min before the PCI, followed by local delivery of argatroban into the dilated site using a Dispatch™ catheter, and the postoperative treatment of intravenous infusion of argatroban for 4 h.

according to consecutive sealed envelopes; the control group (n=35) underwent a conventional method of POBA, and the argatroban group (n=35) had the addition of local delivery of argatroban. The exclusion criteria were (1) more than 80 years old or less than 20 years old, (2) a target lesion in a non-protected left main coronary artery, (3) a total occlusive lesion equal to TIMI 0-1 flow, (4) a severely calcified lesion, (5) a diffuse lesion, (6) a target vessel with severe proximal tortuosity, (7) a lesion that restenosed more than once, (8) a bypass graft vessel, (9) an indication for a new device (eg, directional coronary atherectomy, stent, rotational atherectomy or laser ablation), (10) poor left ventricular function (ejection fraction <40%), (11) patients receiving warfarin, (12) patients receiving an intravenous infusion of heparin, (13) a history of gastrointestinal bleeding, thrombocytopenia, or coagulopathy, (14) a history of stroke within the preceding 3 months, (15) acute myocardial infarction within the previous month, (16) patients undergoing thrombolysis within the past 24 h, (17) pregnancy, and (18) other major illness including renal failure and liver dysfunction. Informed consent was obtained from each patient.

PCI Procedure and Adjunctive Therapy

Coronary angiography was performed using the Judkins method, and a bolus of 5,000–8,000 IU heparin was given intravenously after vascular access had been established. In the control group, the POBA was performed in a standard way with a bolus injection of heparin (50 U/kg per h) during the procedure, followed by an infusion of heparin (25 U/kg per h) for 4 h after the POBA. In the argatroban group, an intravenous infusion of argatroban was given (1 μg/kg per min) 30 min before POBA, followed by the local delivery of argatroban (10 mg/20 min) into the dilated site using the Dispatch™ catheter (SIMED Life Systems) after the successful POBA. Postoperatively, the patients received an intravenous infusion of argatroban (1 μg/kg per min) for 4 h (Fig 1). All patients received both Ca antagonist and 81–162 mg of aspirin before the POBA until the follow-up angiography. In addition, β-blockers, long-acting isosorbide dinitrates or nicorandil was administered at the discretion of the treating physician before the POBA until the follow-up coronary angiography. Clinical success of the POBA was defined as angiographic success (residual stenosis <50%) without a major complication (death, myocardial infarction, or emergency coronary-artery bypass surgery) during hospitalization.

Quantitative Coronary Angiographic Analysis

All angiograms were analyzed by a computer-assisted system of quantitative coronary angiographic analysis (QCA; Cardiovascular Measurement System Ver. 3.0

Table 1 Baseline Clinical Characteristics of the Study Patients

	Control group (n=35)	Argatroban group (n=35)	p value
Age (years)	61±8	61±8	NS
M/F	29/6	27/8	NS
Risk factors			
BMI	23.6±2.0	23.5±2.6	NS
Diabetes mellitus	18	12	NS
Hypertension	19	23	NS
Total cholesterol (mg/dl)	185±32	198±36	NS
Prior MI	13	8	NS
Ejection fraction (%)	57±10	58±13	NS
Diseased coronary vessels			
1-vessel disease	23	23	NS
2-vessel disease	11	12	
3-vessel disease	1	0	
Target vessel			
LAD/LCX/RCA	18/13/4	16/16/3	NS

BMI, body mass index; MI, myocardial infarction; LAD, left anterior desending artery; LCX, left circumflex artery; RCA, right coronary artery.

Table 2 Baseline Lesion Characteristics of the Study Patients

	Control group (n=35)	Argatroban group (n=35)	p value
ACC/AHA classfication			
A	5	5	
B	29	29	NS
C	1	1	
De novo lesion	32 (94%)	28 (80%)	NS
Reference vessel diameter (mm)	2.89±0.46	2.89±0.39	NS
Minimal lumen diameter (mm)	0.86±0.24	0.83±0.19	NS
Lesion length (mm)	6.00±3.69	5.25±3.77	NS
Lesion characteristics			
Eccentricity	29 (83%)	27 (77%)	NS
Calcification	12 (34%)	16 (46%)	NS
Ostial lesion	4 (11%)	5 (14%)	NS
Proximal tortuosty	2 (6%)	1 (3%)	NS
Angled lesion	4 (11%)	4 (11%)	NS
Bifurcation	7 (20%)	6 (17%)	NS

(CMS), Medical Imaging Systems Inc, Leiden, the Netherlands). CAG was performed before, immediately after, and 3 months after the POBA (follow-up) as described in detail elsewhere[19] All angiographic analyses were performed in a blinded fashion by an experienced physician. The % diameter stenosis (%DS) and minimal lumen diameter (MLD) of the target lesion were determined quantitatively. The diameter of a Judkins catheter was measured using a precision micrometer (No. 293-421-20; precision 0.001 mm, Mitutoyo Co, Kawasaki, Japan) to obtain a calibration factor in the 'Free French' mode in the image calibration of the CMS program. The calibration factor (CF) was adjusted between 0.08 and 0.1 mm/pixel using digital zoom according to the CMS manual[20] The complex edit mode (gradient field transform: GFT) was used in the case of a complex lesion, as described in detail elsewhere[21]

Angiographic restenosis after POBA was defined as a %DS greater than 50% on the follow-up angiogram. Clinical restenosis was defined as the recurrence of ischemia and/or target lesion revascularization within the period before the follow-up angiography.

Endpoints

The following endpoints were prospectively defined. Restenosis was the primary endpoint. Secondary endpoints included death, acute myocardial infarction (symptoms, ECG changes, and creatine kinase >twice the upper normal limit) and coronary revascularization (coronary bypass surgery, or repeated POBA and/or coronary stenting). Repeat revascularization of the target lesion (target lesion revascularization) was defined as angioplasty or bypass surgery performed because of restenosis of the target lesion in association with recurrent angina, objective evidence of myocardial ischemia, or both. The principal safety endpoints were abrupt vessel closure, stroke, major bleeding, or the need for vascular surgery. Major bleeding was defined as intracranial hemorrhage or overt bleeding associated with a decrease in hemoglobin of more than 5 g/dl.

Statistical Analyses

The data are presented as mean±SD (standard deviation). Differences in angiographical parameters (%DS and MLD) between the 2 groups before POBA, immediately after all procedure and during the follow-up were compared by unpaired t-test. Statistical comparisons of differences in categorical data between the 2 groups were performed using the chi-square test. Differences were considered significant when $p<0.05$. The clinical follow-up analyses were performed on an intention-to-treat basis and on-treatment-analyses. Moreover, angiographic follow-up analyses were performed using on-treatment-analyses.

Table 3 In-Hospital Outcomes of the Study Patients

	Control group (n=35)	Argatroban group (n=35)	p value
Stent required (%)	3 (8.6)	3 (8.6)	NS
Acute occlusion (%)	0 (0)	1 (2.8)	NS
Additional ballooning (%)	0 (0)	1 (2.8)	NS
Angiographical nonsuccess (%)	1 (2.8)	0 (0)	NS
Acute myocardial infarction (%)	0 (0)	0 (0)	NS
Emergency CABS (%)	0 (0)	0 (0)	NS
Death (%)	0 (0)	0 (0)	NS

CABS, coronary artery bypass surgery; MI, myocardial infarction.

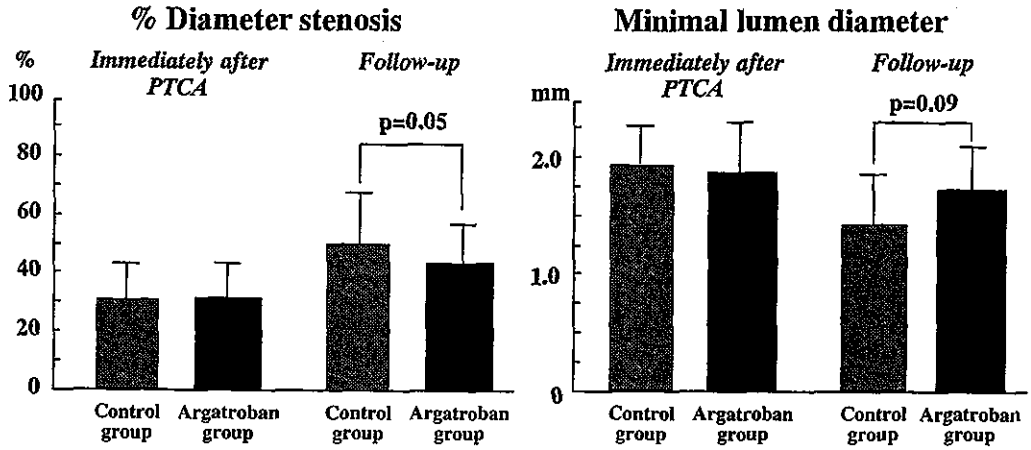

Fig 2. There were no significant differences between the 2 groups in % diameter stenosis or minimal lumen diameter immediately after PCI. Angiographic parameters including % diameter stenosis and minimal lumen diameter were marginally better in the argatroban group than in the control group at follow-up.

Results

Patient Population (Fig 1)

Four patients in the argatroban group were excluded from the follow-up CAG; 1 underwent stent implantation because of a major coronary dissection before the local delivery of argatroban, 1 had an abrupt vessel closure during the local delivery of argatroban, 1 required additional ballooning and stent implantation, and 1 refused to undergo the follow-up CAG with negative exercise thallium-201 stress imaging. Four patients in the control group were also excluded from the follow-up CAG: 3 required stent implantation because of major coronary dissection after the balloon angioplasty, and 1 had residual %DS >50% (angiographically unsuccessful). During the course of the study, 2 patients died suddenly (control 1, argatroban 1) before the follow-up angiography; the 1 in the argatroban group had cardiac sudden death after balloon angioplasty on day 60 (the patient had an old myocardial infarction with left ventricular dysfunction) and the patient in the control group died suddenly on day 60 after the balloon angioplasty (suspected rupture of a thoracic aortic aneurysm). In total, 10 patients (5 in each group) were excluded from the follow-up angiography.

Baseline Clinical and Lesion Characteristics

Tables 1 and 2 summarize the baseline clinical and lesion characteristics; there were no significant differences between the 2 groups in this study.

In-Hospital Outcome

The in-hospital outcomes are summarized in Table 3. An acute occlusion in the treated segment during the local delivery of argatroban using a Dispatch™ catheter was observed in 1 patient, requiring implantation of a Palmaz-Schatz stent. There were no major complications during the procedure in either group.

Quantitative CAG Analyses at Follow-up

Fig 2 compares the results of the angiographic analyses between the 2 groups. There were no significant differences between the 2 groups in %DS (Control group: 30.9±10.9%, Argatroban group: 31.7±9.6%) or MLD (Control group: 1.95±0.3 mm, Argatroban group: 1.92±0.35 mm) immediately after procedure. However, after 3 months, the angiographic parameters of %DS (Control group: 51.3±16.2%, Argatroban group: 43.5±14.6%) and MLD (Control group: 1.36±0.46 mm, Argatroban group: 1.57±0.47 mm) were marginally better in the argatroban group than in the control group (p=0.05 and p=0.09, respectively). The mean difference in coronary MLD (net gain) between the post-procedure and follow-up angiograms was 0.51±0.44 mm in the control group, and 0.72±0.50 mm in the argatroban group (p=0.09).

Restenosis Rates, Target Lesion Revascularization, and Clinical Follow-up Data

Fig 3 compares the restenosis rates in the 2 groups. The lesion restenosis (%DS >50%) rates were 27% in the argatroban group and 56% in the control group (p=0.02). The clinical restenosis rates were 14% in the argatroban group and 37% in the control group (intention-to-treat analysis; Table 4, p=0.03). The target lesion revascularization rates were 14% in the argatroban group and 34% in the control

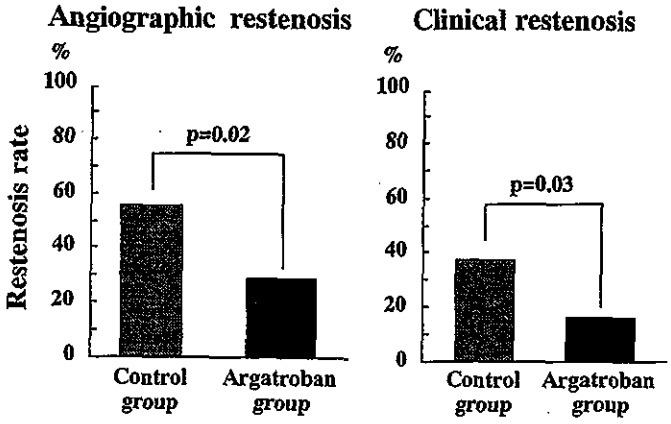

Fig 3. Angiographic restenosis occurred in 5 of the 30 patients in the argatroban group (27%) and 12 of the 30 patients in the control group (56%). Clinical restenosis occurred in 5 of the 35 patients in the argatroban group (14%) and 13 of the 35 in the control group (37%).

Table 4 Clinical Outcome at Follow-up of the Study Patients

	Control group (n=35)	*Argatroban group (n=35)*	*p value*
Clinical restenosis	*13**	*5*	*0.03*
Target vessel revascularization	*12*	*5*	*0.05*
Vasospastic angina	*0*	*1*	*NS*
Myocardial infarction	*0*	*0*	*NS*
Death	*1*	*1*	*NS*
Any clinical event	*14*	*7*	*0.07*

Clinical restenosis included recurrence of ischemia and/or angina and target vessel revascularization.
**Includes one case of recurrence of ischemia (silent) without target vessel revascularization.*

Table 5 Clinical Outcome at Follow-up of the Study Patients According to on-Treatment Analysis

	Control group (n=31)	*Argatroban group (n=32)*	*p value*
Clinical restenosis	*12**	*5*	*0.03*
Target vessel revascularization	*11*	*4*	*0.06*
Vasospastic angina	*0*	*1*	*NS*
Myocardial infarction	*0*	*0*	*NS*
Death	*1*	*1*	*NS*
Any clinical event	*13*	*7*	*0.08*

Clinical restenosis included recurrence of ischemia and/or angina and target vessel revascularization.
**Includes one case of recurrence of ischemia (silent) without target vessel revascularization.*

group (intention-to-treat analysis; Table 4, p=0.05). Moreover, Table 5 shows the clinical outcome at follow-up of the study patients on-treatment-analysis. Seven cases (6 stent implantations and 1 unsuccessful procedure during initial angioplasty) were excluded in Table 5 according to on-treatment-analysis. The clinical restenosis rates at follow-up were 17% (n=5) in the argatroban group and 40% (n=12) in the control group according to on-treatment-analysis after exclusion of 10 cases (n=30, respectively; p=0.04). The details of those 10 cases are as follows: 6 stent implantations during procedure, 1 unsuccessful procedure, 2 deaths, and 1 refusal of follow-up CAG.

Discussion

Previous and Present Trials Regarding the Prevention of Restenosis

No definitively effective prevention of restenosis by systemic administration of drugs has been observed in previous clinical trials. Several types of drug therapy, such as anticoagulants (heparin, warfarin) and antiplatelet therapy (aspirin, dipyridamole, ticlopidine, prostacyclin, and thromboxane A2 inhibitor), fish oil, and steroids have failed to reduce the restenosis rate in most clinical trials[22–24] Recently, trapidil and cholesterol-lowering agents have been shown to be promising in preventing restenosis after coronary angioplasty[25] but patients must take these drugs for several months after angioplasty.

In contrast, coronary stenting has been shown to be effective in preventing restenosis after coronary angioplasty[26,27] and the drug eluting stent has been developed in recent years[28] Nevertheless, adjunctive anticoagulation and/or antiplatelet therapy is required for 1 month after coronary stenting, resulting in occasional bleeding complications. Accordingly, a new procedure with a low rate of adverse effects and no need for adjunctive therapy after discharge has been sought. In the present randomized, controlled study, local delivery plus intravenous infusion of argatroban reduced both the angiographic and clinical restenosis rates after coronary angioplasty. There was no increase in bleeding risk with the argatroban treatment. The restenosis rate in the argatroban group in this trial (27%) was similar to that in the stent group of the STRESS trial (32%; NS)[27] despite the fact that the reference vessel diameter was smaller (2.89±0.39 mm) than that in the STRESS trial (3.03±0.42 mm; p=0.07). The restenosis rate in the

control group in the present study was similar to that in the control group of the CAVEAT trial (56% vs 57%)?[29]

Mechanism of Restenosis and Thrombin Activation

The mechanism of restenosis after PCI is considered to be a healing process after a balloon injury. Immediately after arterial injury with a balloon catheter, many factors lead to the activation of medial smooth muscle cells (SMC), but there are 3 major ones. First, elastic recoil is a pivotal factors after mechanical trauma to the abnormal vessel wall and stretching of the normal vessel wall (ie, arterial remodeling). Second, the formation of thrombus on the intimal surface and inside the disrupted plaque is an important part of the restenosis process. The intensity of thrombus formation could serve to reduce the initial gain in lumen both by adding to the plaque mass and by elaborating more growth factors?[30] Third, the most intense interest has been on the impact of mitogenic factors (basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), and SMC-derived growth factor (SDGF)) released by platelets, monocytes, and by components of the intact parts of the vascular wall, including the SMC. In vitro and in vivo studies have shown that injury to the endothelium and the vessel wall causes increased thrombin production?[31] Thrombin, in particular, may play a significant role in the initiation of the restenosis process, because the regulation of these growth factors has been reported to be modulated by thrombin via a thrombin receptor?[32,33] Moreover, thrombin activates a variety of vascular and inflammatory cell types that promote wound healing![10,32] Thus, the inhibition of the initial thrombin activation may exert a potent preventative effect on restenosis after POBA.

Direct Thrombin Inhibitors and Restenosis

The direct thrombin inhibitors, such as r-hirudin, hirulog, hirugen and D-Phe-Pro-Arg-chloromethylketone (PPACK), are expected to reduce the restenosis rate after PCI![11,34] and several relevant experimental studies have been performed in recent years. Rogasta et al reported that the 2-h systemic infusion of hirudin failed to reduce cell proliferation within the first 7 days, whereas the 2-h infusion of hirulog improved the late angiographic luminal dimensions and reduced the cross-sectional area narrowing by plaque in rabbits compared with heparin controls after angioplasty![12] They suggested that (1) hirudin inhibits cellular migration rather than proliferation, and (2) hirudin reduces mural thrombosis, resulting in less thrombus incorporation into the plaque. However, Serruys et al reported that the systemic administration of r-hirudin failed to reduce restenosis in a clinical study (HELVETICA study)?[35] This discrepancy between the experimental study (Rogasta et al[12]) and the clinical study (Serruys et al[35]) may be explained by a difference in the local concentration of hirudin at the target lesion. Accordingly, it is expected that the local delivery of a high concentration of a direct thrombin inhibitor using a drug delivery device would reduce restenosis without increasing adverse effects in the clinical setting. However, there has not a previous clinical prospective randomized trial using a direct thrombin inhibitor and a local delivery device for preventing restenosis after angioplasty.

The present study has demonstrated that the intracoronary local delivery of argatroban, in addition to a 4-h intravenous infusion, prevents restenosis following POBA. Argatroban has been reported to inhibit platelet activation by fibrin- or clot-incorporated thrombin more effectively than does hirudin![16] The reason that both local delivery and continuous intravenous infusion of argatroban were used in the present study was to inhibit thrombin activity, which may increase immediately after angioplasty before the local delivery of argatroban, because there was a time delay (approximately 10 min) between the first balloon inflation and the local delivery of argatroban (thrombin receptors have been reported to appear on a SMC within a few min after balloon injury[12]). The present findings, together with the report of Rogasta et al![13] suggest that direct thrombin inhibition may successfully inhibit cell migration in the initiation of restenosis in human patients.

Local Drug Delivery Device

Several local delivery balloon catheters have been designed. The double-balloon catheter was the first percutaneous drug delivery device. Other drug delivery devices such as the Wolinsky perforated-balloon catheter, a microporous balloon, a channel catheter, and the Transport coronary angioplasty catheter have been developed since then. More recently, the drug delivery devices known as the Infusasleeve™, a hydrogel-coated balloon, and the Dispatch™ catheter have become available?[36] The hydrogel-coated balloon does not have a perfusion port to support distal blood flow during balloon inflation. Imanishi et al reported that the local delivery of argatroban using a hydrogel-coated balloon reduced intimal thickening after balloon injury in an experimental study?[37] The Dispatch™ catheter consists of an over-the-wire, non-dilatation catheter with a spiral inflation coil and a perfusion port on its distal tip. There are several advantages of this system for the drug delivery. First, this device allows distal coronary perfusion during balloon inflation for a sufficiently longer time compared with other drug delivery catheters. Second, this system makes it easier to deliver the drug than a hydrogel-coated balloon catheter, because in the case of the hydrogel-coated balloon, the drug must first penetrate the hydrogel-balloon surface. Third, the pharmacokinetic validity of the local delivery of argatroban using a Dispatch™ catheter has been established. Anabuki et al confirmed that the local delivery of argatroban using a Dispatch™ catheter resulted in the intramural deposition of high concentration argatroban without any arterial damage?[38] This new device has been used for the prevention of reocclusion after revascularization in patients with acute myocardial infarction and unstable angina pectoris![18,39] However, there are no other clinical reports on the prevention of restenosis using the Dispatch™ catheter except for one small non-randomized trial![10] Thus, this is the first prospective randomized controlled trial using the Dispatch™ catheter and argatroban to prevent restenosis following POBA. Moreover, it is expected that these local delivery devices may be available not only for direct thrombin inhibitor but also gene therapy in the future?[40]

Study Limitations

First, it is unclear whether the local delivery of argatroban using a Dispatch™ catheter is effective in patients with small vessels (<2.5 mm). Second, this study was designed as an open-label randomized trial in the light of safety concerns. Although a double-blind design may be better, it is not easy to use a specific device such as the Dispatch™ catheter in a double-blind manner. Because no

obvious benefit of long-term inflation in preventing restenosis was found in a previous study;[41] the long-term inflation (20 min) with the Dispatch™ catheter is unlikely to be responsible for the significant reduction of restenosis in the present study. Third, this trial was performed at a single center, with a small number of patients. Further study is necessary with a larger number of patients in a double-blind, randomized, multicenter trial with a placebo group (local delivery of normal saline using a Dispatch™ catheter). We are now planning to conduct such a trial in Japan. Fourth, the effect of the exclusively local delivery of argatroban remains undetermined, because postoperative intravenous infusion of argatroban was combined with the intracoronary local delivery in the present study. Further study is necessary to assess the 'pure' efficacy of the local delivery of argatroban. Moreover, further study is necessary to assess the efficacy for stenting lesions in the present stenting era. Final, the present study did not evaluate local delivery direct pressure, although it is reported that high, local delivery pressure is a key determinant of vascular damage and intimal thickening.[42] Further study is needed to examine the local drug delivery pressure during infusion of argatroban in the clinical setting.

Conclusions

The local delivery of argatroban using a Dispatch™ catheter was observed to be safe and effective in preventing restenosis after balloon angioplasty.

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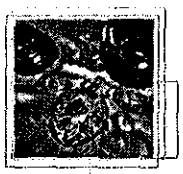

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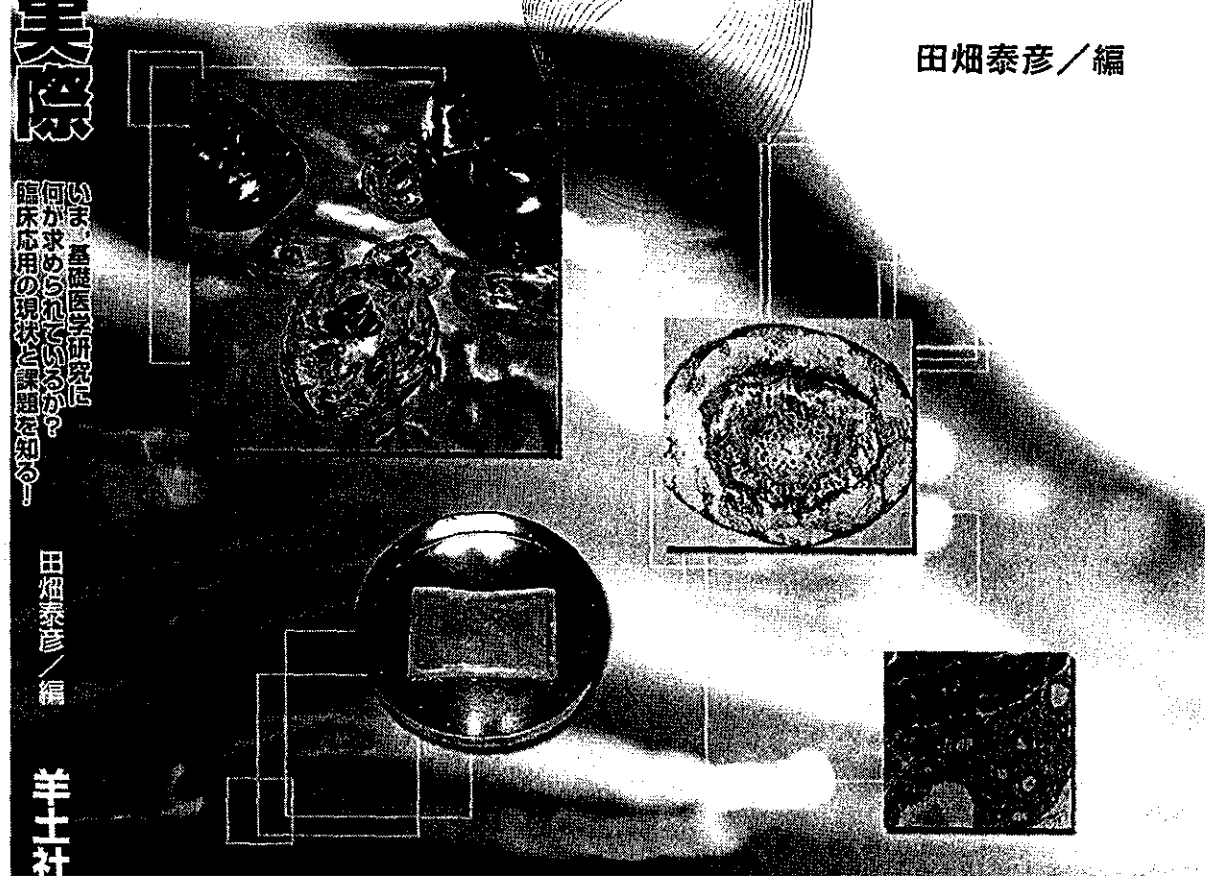

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3. 遺伝子による血管新生

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1994年に行われた循環器領域における遺伝子治療開始以来，次々にその有効性が報告されている．これら循環障害に対しての遺伝子治療は遺伝子治療全体のなかでもっとも良好な結果が得られているといっても過言ではないと思われる．近年，nakedプラスミドや骨髄単核球投与による血管新生療法も臨床において開始されており，増え続ける虚血性疾患に対しての新たな治療法としての位置を確保しつつある．本稿においては，新たな治療法として行われてきている遺伝子投与による血管新生療法の現況を概説し，われわれが開発中の生分解性ゼラチンを用いた遺伝子導入法とCell/Gene Hybrid Therapyについても解説する．

再生医療の現状

Folkmanにより腫瘍発育に血管新生因子による新生血管が関与していることが示されて以来[1]，分子生物学の発展に伴い血管形成の機序が徐々に明らかになってきた．悪性新生物における血管新生の抑制，または虚血に対しての血管新生療法の可能性を示唆する研究報告がなされるなか，血管新生促進因子による血管新生（再生）を得ることで虚血性疾患の治療を行う「治療的血管新生（therapeutic angiogenesis)」の概念が誕生した[2]．そして，1994年に米国タフツ大学のJeffrey M. Isnerらにより，循環器領域における世界初の遺伝子治療が行われた[3]．その後も，それ以外の血管新生促進因子を用いた治療的血管新生の検討が行われてきている．

1. 血管新生促進因子

種々の血管新生促進因子による血管新生（angiogenesis）あるいは血管形成（vasculogenesis）が報告されている（表1）．以下にその主なものについて述べる．

1）FGF（fibroblast growth factor：線維芽細胞増殖因子）

FGFファミリーはヘパリンに親和性の高いポリペプチドであり，aFGF（acidic FGF：酸性FGF=FGF-1），bFGF（basic FGF：塩基性FGF=FGF-2），int-2（FGF-3），hst-1（FGF-4），FGF-5がある．FGFは内皮細胞のみでなく線維芽細胞や平滑筋細胞を増殖させる働きがある．このことは毛細血管のみでなく細小動脈の新生をきたす可能性があるが，増殖性病変形性の可能性もある．aFGF，bFGFに関しては分泌シグナルが付いていないためにその分泌機序は詳細が不明である．一方，int-2，hst-1はシグナルペプチドをもち分泌されるタンパク質であり，VEGF産生を促進するhst-1/FGF-4は血管新生療法においてより有効である可能性があり狭心症に対しての臨床治験でも有効性が報告されている[4]．また，FGF-5は脈絡膜血管新生に関与しているとの報告がある．

2）VEGF（vascular endotherial growth factor：血管内皮増殖因子）

1つの遺伝子から5種類のアイソフォーム（121，145，165，189，206アミノ酸）が産生され，PDGF

(platelet derived growth factor：血小板由来増殖因子)-Aあるいは-Bと似た構造をしている．*in vitro*では内皮細胞の増殖促進，アポトーシスの抑制，*in vivo*では血管新生，血管透過性はもちろん管腔形成促進，内皮細胞の遊走，凝固線溶系タンパク質の産生，細胞接着分子の内皮細胞上への発現等を誘導する．VEGFはシグナルペプチドをもつため，分泌されパラクリン的に血管内皮細胞に働き，低酸素状態に反応して働くという大きな特徴がある．これは，VEGFの転写開始点よりも上流に結合するHIF-1α（hypoxia-inducible factor-1α：低酸素誘導因子1α）を介しての転写亢進が関与している．VEGF関連遺伝子群としてPlGF（placenta growth factor：胎盤由来増殖因子），VEGF-B, -C, -D, -Eも発見されその効果について検討がなされている．最近，生物学的活性の強い$VEGF_{165}$に特異的に結合するneuropilin-1（NP-1）が報告された[5]．これは単独では活性を示さないが，内皮細胞に発現させるとVEGF受容体に対する結合が約10倍に上昇し，活性も同程度上昇することが示された．VEGFは内皮細胞に限局的に働くが，透過性の増大による浮腫をきたす例が少なくない．

3）HGF（hepatocyte growth factor：肝細胞増殖因子）

HGFは，肝臓の再生因子として発見されたが，腎臓，肺，消化管そして血管等さまざまな臓器に関与している．HGFは典型的なシグナル配列をもつため細胞から分泌され，，受容体であるc-Metが内皮細胞に存在することから，VEGFと同様に血管平滑筋細胞には影響を与えず，内皮細胞のみを増殖させることが明らかになっている[6]．HGFは虚血の状況下においてはその発現は著明に低下しており，VEGFとは異なる．しかし，受容体であるc-Metの発現は増加しており，HGFを遺伝子導入することによりその不足分を補うことが可能となり，結果としてVEGFと同等な治療効果を得ることができるとされている．現在，臨床においての投与が開始されており，その有効性が報告されている．副作用としての浮腫はVEGFと異なり報告されていない．

4）その他の血管新生促進因子

プロスタグランジン（PGE_1, PGE_2）は血管拡張作用と血管新生作用をもち，化学的安定化を図ったプロドラッグの報告がある[7]．

炎症性サイトカインにはIL-1/6/8，TNF-α，イン

略　語

FGF：fibroblast growth factor（線維芽細胞増殖因子）
VEGF：vascular endotherial growth facto（血管内皮増殖因子）
PDGF：platelet derived growth facto（血小板由来増殖因子）
HIF-1α：hypoxia-inducible factor-1α（低酸素誘導因子1α）
PlGF：placenta growth factor（胎盤由来増殖因子）
NP-1：neuropilin-1
HGF：hepatocyte growth factor（肝細胞増殖因子）
PA：plasminogen activator
MMP：matrix metalloproteinase
PECAM-1：platelet endotherial cell adhesion molecule-1
G-CSF：granulocyte colony stimulating factor（顆粒球コロニー刺激因子）
GM-CSF：granulocyte-macrophage colony stimulating factor（顆粒球コロニー刺激因子）
PGE_1, E_2：prostaglandin E_1, E_1
PD-ECGF：platelet derived-endothelial cell growth factor（血小板由来内皮細胞増殖因子）
TNF-α：tumor necrosis factor-α（腫瘍壊死因子-α）
EGF：epidermal growth factor（上皮成長因子）
TGF-β：transforming growth factor-β（形質転換増殖因子）
PAF：platelet-activating factor（血小板活性因子）
ECK：epitherial cell kinase