Single-Dose Intravenous Administration of Recombinant Human Erythropoietin Is a Promising Treatment for Patients With Acute Myocardial Infarction

– Randomized Controlled Pilot Trial of EPO/AMI-1 Study –

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Background: Erythropoietin (EPO) has been found to have anti-apoptotic and tissue protective effects on the myocardium. The aim of the present pilot study was to observe the safety and efficacy of EPO administration for patients with acute myocardial infarction (AMI).

Methods and Results: Patients admitted with AMI had all undergone successful percutaneous coronary intervention (PCI). Patients were randomly assigned to 2 groups (control and EPO groups), and given 12,000 IU EPO iv or saline after PCI. The primary endpoints were the difference between the acute phase and chronic phase (6 months after the attack) regarding left ventricular function as measured on electrocardiogram-gated single-photon emission computed tomography. Thirty-six patients (control 16, EPO 20) were eligible for analysis. Left ventricular ejection fraction (LVEF) significantly increased in the EPO group (from $51.0 \pm 19.6\%$ to $58.5 \pm 15.0\%$, $P=0.0238$), but not in the control group. Further analysis was separately undertaken in patients with occlusion in the left anterior descending artery (LAD) and others (non-LAD). LVEF was $<50\%$ in most patients in the LAD subgroup, and LVEF significantly increased in the EPO group (37.5 ± 13.0 to 52.7 ± 15.8 , $P=0.0049$), but not in the control group. EPO administration did not trigger any adverse clinical events.

Conclusions: EPO administration is a promising treatment for AMI. (*Circ J* 2010; **74**: 1415–1423)

Key Words: Acute myocardial infarction; Erythropoietin; Single-photon emission computed tomography

Reperfusion therapy involving percutaneous coronary intervention (PCI) has been utilized for the treatment of patients with acute myocardial infarction (AMI). Although the application of PCI has improved the prognosis of AMI, the disease is still serious, and some patients suffer from insufficient cardiac function. Left ventricular ejection fraction (LVEF) is one of the most important prognostic factors for survivors of MI.¹ Previous randomized trials introducing new therapeutic options for patients with AMI such as administration of β -blockers and angiotensin-converting enzyme (ACE) inhibitors have shown reduced morbidity and mortality with an LVEF increase of $>3\%$.^{2,3} Despite previous

intensive research, no drug has exceeded the effect of reperfusion by PCI to reduce infarct size in patients with AMI.⁴

In addition to hematopoietic activity, erythropoietin (EPO) has been found to have anti-apoptotic and tissue-protective effects on the myocardium in animal models. Independent of hematopoietic activity, administration of EPO improved cardiac contractility in post-hypoxic mice,⁵ repressed cell death of the myocardium in a rabbit AMI model,⁶ expressed an acute cardioprotective effect in rat ischemia–reperfusion injuries,⁷ showed a preventive effect on cardiac dysfunction in doxorubicin-induced cardiomyopathy in mice,⁸ and protected the myocardium in rats with experimental autoimmune

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myocarditis^{9,10} via protein kinase C and phosphatidylinositol 3-kinase signaling directly¹¹ and neovascularization by EPO indirectly.¹²

Clinical trials of EPO administration have been carried out to treat stroke and coronary artery disease. Administration of 33,000 IU recombinant human EPO (rhEPO: approximately 0.2 IU/ng) once a day for 3 consecutive days was safely and beneficially tried for acute stroke.¹³ In contrast, single-dose administration of 300 mg darbepoietin, a long-acting analog of rhEPO (the hematopoietic activity is much less than 0.2 IU/ng), was feasible and safe, but not beneficial for AMI.¹⁴ Another trial was also started to estimate the efficacy and safety of a single bolus of 60,000 IU rhEPO for AMI in January 2007.¹⁵

We also planned a clinical trial of single-dose 12,000 IU rhEPO for the treatment of patients with AMI in December 2005. The dose was selected to minimize the thromboactivating effect of EPO while preserving the cardioprotective action. Relatively low-risk AMI patients were enrolled to avoid serious vascular events after EPO administration.

Methods

Participants

The study was initiated in December 2005. We had prospectively enrolled AMI patients with ST-segment elevation ≥ 1 mm on at least 2 limb leads or ≥ 2 mm in consecutive precordial leads. Patients were eligible if they were admitted within 24 h after onset of AMI and had undergone successful PCI for a single infarct-related artery. Patients who achieved successful PCI with Thrombolysis In Myocardial Infarction flow grade 3 reperfusion were randomized into the control or EPO group. Exclusion criteria were previous MI, multi-vessel disease, persistent severe heart failure (Killip class III or IV), cardiogenic shock, age >80 years, advanced hepatic or renal dysfunction (Cre ≥ 2 mg/dl, T-Bil ≥ 3 mg/dl, blood pressure $>140/90$ mmHg at the time of administration of EPO, and a hematocrit level $>60\%$ in male patients or $>55\%$ in female patients after PCI.

Study Protocol

The study was a prospective, randomized, single-blind, placebo-controlled multi-center trial of the Evaluation of the Prospective Observation of Erythropoietin Administration for the Treatment of Acute Myocardial Infarction (EPO/AMI-1 pilot study) within the following hospitals: Niigata University Medical and Dental Hospital, Showa University Hospital, Showa University Fujigaoka Hospital, Niigata Kofu Hospital, and Dokkyo Medical University Hospital. The institutional ethics review board of each hospital approved the study protocol. We obtained written informed consent from patients after explaining the procedures and risks. Randomization was done by a blinded independent statistician, and the study processes were not blinded after randomization. In the EPO group, patients received a single iv administration of saline 100 ml mixed with EPO 12,000 IU (epoetin-beta, Chugai Pharmaceutical, Japan) within 24 h after PCI. In the control group, the patients were injected iv with saline 100 ml. All patients were given heparin iv (10,000–15,000 U/day) according to the standard protocol. Optimal standard treatment after PCI included administration of an aspirin, thienopyridine, lipid-lowering therapy, ACE inhibitor, angiotensin II blocker, β -blocker, and calcium antagonist. We assessed the safety of EPO therapy on the basis of: development of major adverse clinical events (MACE: death, new MI, revasculariza-

tion, admittance to hospital due to aggravation of ischemia or heart failure), in-stent re-stenosis, proarrhythmic effects, EPO-related polycythemia, and thrombosis. All patients underwent follow-up visits every 3 months after enrollment and were assessed for clinical status, safety, adverse reactions, and medication.

The primary endpoint was the improvement of cardiac function: LVEF as a parameter of global left ventricular cardiac function, regional ejection fraction (r-EF) as a parameter of local contraction of an infarcted area, left ventricular end-diastolic volume index (EDVI), left ventricular end-systolic volume index (ESVI), and infarct size measured on electrocardiogram (ECG)-gated ^{99m}Tc-hexakis-2-methoxy-isobutyl isonitrile (MIBI) single-photon emission computed tomography (SPECT). The secondary endpoints were the rate of coronary re-stenosis, frequency of arrhythmia and the changes in brain natriuretic peptide (BNP) from the acute phase to 6 months after the onset of AMI.

Procedure

We performed ECG-gated ^{99m}Tc MIBI SPECT 4 days after PCI as baseline and at 6-month follow up. The ^{99m}Tc-MIBI (600 MBq) was administered at baseline and three-quarters of 740 MBq at 6-month follow up. SPECT image acquisition was performed 60 min after injection of ^{99m}Tc-MIBI. ECG-gated SPECT was acquired after administration of ^{99m}Tc-MIBI at rest. In ECG gating, SPECT data divided into 16 equal intervals was analyzed using pFAST2 software (Fujifilm RI Pharma, Tokyo, Japan), and EDVI, ESVI and LVEF/r-EF were calculated using cardioGRAF software (Fujifilm RI Pharma). The non-gated SPECT image was used to assess the severity of myocardial perfusion abnormalities, and regional uptake and the infarct area was calculated using cardioBull software (Fujifilm RI Pharma). Regional uptake and r-EF were assessed by applying a 17-segment model of the left ventricle according to the standardized myocardial segmentation by the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association.¹⁶ Regional uptake was expressed as the mean uptake count in these segments. Defects at less than the threshold of 60% of peak counts were identified as an infarcted myocardium, and the infarct area was expressed as a percentage of the entire left ventricle involved. SPECT data analysis was performed in a blinded fashion by a single radiologist with no knowledge of the clinical status or medical therapy of patients.

We performed coronary angiography (CAG) and left ventriculography (LVG) at baseline and at 6-month follow-up catheterization. The EDVI, ESVI, and LVEF were calculated in an area-length method from the right anterior oblique view of LVG by another radiologist. Quantitative CAG (QCA) was performed at baseline and 6-month follow up by an independent specialist with a CCIP-310 system (Cathex, Tokyo, Japan). The minimal lumen diameter (MLD) and reference vessel diameter were measured from diastolic frames in single, matched views showing the smallest lumen diameter. Binary restenosis was defined as diameter stenosis $>50\%$ within the stent or in 5-mm segments proximal or distal to the stent at follow up.

The number of premature ventricular complexes per day and the occurrence of non-sustained or sustained ventricular tachycardias were evaluated on Holter monitoring at baseline and 6-month follow up. Plasma BNP levels were measured at baseline (day 14) and 6 months after PCI. CD34-positive cells in peripheral blood were measured at baseline and on

days 4, 7, and 14 after EPO/placebo injection.

Statistical Analysis

Unpaired data were assessed using the Wilcoxon rank-sum test for continuous variables. Paired data were tested using the Wilcoxon signed-rank test. Differences were considered significant for $P < 0.05$. All data are expressed as mean $\pm$ SD. Statistical analysis was undertaken with JMP version 5.0 (SAS Institute, Cary, NC, USA).

Occlusion in the left anterior descending artery (LAD) results in remarkable deterioration of LVEF because of its large territory in the left ventricle, while left ventricular cardiac dysfunction caused by the occlusion of the left circumflex coronary artery or right coronary artery is relatively small. We therefore divided both the control and EPO groups into 2 subgroups and analyzed them to determine the effect of EPO on the malfunctioning left ventricle: infarct-related artery in LAD and those in other arteries (non-LAD) as described in a previous article.¹⁷

Results

Patient Characteristics

Before August 2008, 41 patients were informed concerning the trial, and were randomly allocated to treatment (Figure 1). After randomization, 5 patients were withdrawn from analysis. The final total included 16 controls and 20 patients in the EPO group. Table 1 shows the patients' baseline characteristics. All the patients received optimum post-infarction medical treatment. There were no significant differences between the control and EPO groups regarding patient background at

baseline.

Blood Cell Count and Serum EPO Levels

Table 2 lists the blood cell count and CD34-positive cells (%) in peripheral blood during the first 2 weeks. Both reticulocyte (%) and CD34 (%) reached their peak on day 7, but there was no significant difference between the 2 groups. No patients had polycythemia.

Among the 36 patients, serum EPO levels were measured in 18 patients on enzyme-linked immunosorbent assay from Mitsubishi Chemical Medience (Tokyo, Japan; Table 3). In the control group, EPO levels altered within the normal range, but they significantly increased in the 24 h after PCI. The correlation of levels of serum EPO and hemoglobin (Hb) were also analyzed. R-square of the correlation was 0.0867 (before), 0.2539 (24 h), 0.1752 (48 h) and 0.5990 ($P < 0.05$, 72 h). Thus it was plausible that intrinsic secretion of EPO increased the serum EPO level within 24 h, and secondary anemia caused by medical intervention influenced the EPO levels thereafter. In contrast, serum EPO levels increased as high as 1 IU/ml throughout the 24 h after EPO administration in most patients, and that might be a sufficient concentration to protect the myocardium from ischemia-reperfusion injury.

Effect of EPO on Cardiac Function on Tc-MIBI Scintigraphy

At 6-month follow up after randomization, LVEF increased significantly in the EPO group (from $51.0 \pm 19.6\%$ to $58.5 \pm 15.0\%$, $P = 0.0238$), but not in the control group (from $47.2 \pm 16.2\%$ to $51.3 \pm 18.2\%$, $P = 0.2292$) as shown in Figure 2 and Table 4. There was no significant difference, however, in the LVEF increment at 6-month follow up (Δ LVEF) between the

Table 1. Patient Characteristics			
	Control (n=16)	EPO (n=20)	P value
Age (years) (mean±SD)	59.4±13.9	62.5±8.0	NS
Male (%)	14 (87.5)	16 (80.0)	NS
BL (cm)	162.3±8.4	163.9±7.9	NS
BW (kg)	61.7±10.5	63.4±11.3	NS
BMI	23.3±2.5	23.4±2.9	NS
Risk factors			
Hypertension (%)	6 (37.5)	13 (62.5)	NS
Hyperlipidemia (%)	8 (50.0)	8 (40.0)	NS
Diabetes mellitus	4 (25.0)	4 (20.0)	NS
Smoking (%)	12 (75.0)	14 (70.0)	NS
PAD (%)	0 (0)	1 (5.0)	NS
Reperfusion time (min)	176.4±160.1	173.3±118.9	NS
Max CK, IU/L	4,325.9±3,649.3	3,853.3±2,271.1	NS
Max MB, U/L	422.6±353.8	343.5±260.6	NS
PCI procedure			NS
Bare metal stent	11 (68.8)	17 (85.0)	
Drug-eluting stent	4 (25.0)	3 (15.0)	
Aspiration only	1 (6.3)	0 (0)	
Infarct-related artery			NS
LAD	9 (56.3)	11 (55.0)	
Cx	1 (6.3)	1 (5.0)	
RCA	6 (37.5)	8 (40.0)	
Medication			
ACEi/ARB (%)	14 (87.5)	20 (100)	NS
Ca antagonist (%)	5 (31.3)	6 (30.0)	NS
β-blocker (%)	9 (56.3)	12 (60.0)	NS
Statin (%)	14 (87.5)	19 (95.0)	NS
TIMI flow grade before PCI			
Grade 0	9 (56.3)	10 (50.0)	NS
Grade I	5 (31.3)	6 (30.0)	
Grade II	1 (6.3)	4 (20.0)	
Grade III	1 (6.3)	0 (0)	

Data are mean±SD.

EPO, erythropoietin; BL, body length; BW, body weight; BMI, body mass index; PAD, peripheral artery disease; CK, creatine kinase; PCI, percutaneous coronary intervention; LAD, left anterior descending artery; Cx, left circumflex coronary artery; RCA, right coronary artery; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; TIMI, Thrombolysis In Myocardial Infarction.

Table 2. Blood Cell Count				
	Baseline	Day 4	Day 7	Day 14
RBC (10⁴ cells/μl)				
Control	420.7±77.1	405.9±63.1	412.6±62.1	438.8±57.6
EPO	417.0±48.8	404.6±37.8	420.6±29.7	429.5±33.3
Hb (g/dl)				
Control	12.9±2.3	12.3±2.0	12.6±1.9	13.1±1.6
EPO	13.3±1.6	12.9±1.3	13.3±1.1	13.5±1.2
Ht (%)				
Control	38.2±6.1	37.1±5.3	37.4±5.1	39.3±4.4
EPO	38.9±5.1	38.0±4.0	39.2±2.9	40.3±3.5
Reticulocyte (%)				
Control	11.9±3.8		15.5±5.0	11.0±3.9
EPO	11.9±3.0		19.7±7.6	11.9±8.3
CD34+ cells (10⁻³ %)				
Control	9.1±7.9	14.4±11.3	18.2±10.4	17.5±7.9
EPO	9.7±5.8	13.6±4.9	23.4±9.6	16.0±5.7

Data are mean±SD. There were no significant differences among the 2 groups.

RBC, red blood cell; Hb, hemoglobin; Ht, hematocrit. Other abbreviation see in Table 1.

	Control group (n=9)		EPO group (n=9)	
	Mea D	P value	Mean±SD	P value
Before PCI	13.8±2.6		19.4±4.9	
24 h	27.0±7.4	0.0078	1,365.3±589.0	0.0312
48 h	23.7±11.5	NS	161.4±178.6	NS
72 h	21.4±11.8	NS	61.2±49.4	0.0078
14 days	13.5±2.2	NS	21.0±4.2	NS
6 months	17.7±5.4	NS	19.4±6.4	NS

Normal range: 9.1–32.8 mIU/ml. P: comparison with before (Wilcoxon signed rank test).

Abbreviation see in Table 1.

Figure 2. ^{99m}Tc -MIBI scintigraphy in the post-myocardial infarction early phase (0) and at 6-month follow up (6M) of global left ventricular ejection fraction (LVEF), regional ejection fraction (r-EF), and infarct-related arteries. (A) LVEF increased significantly in the erythropoietin (EPO) group ($P=0.0238$) at 6-month follow up, but not in the control (Cont.) group ($P=0.2292$). Both the control and EPO groups were divided into 2 subgroups and analyzed. (B) In the left anterior descending artery (LAD) subgroup LVEF was $<50\%$ in most patients, and increased significantly in the EPO group ($P=0.0049$), but not in the control group ($P=0.3594$). (C) In contrast, in the left circumflex artery or right coronary artery (ie, non-LAD) group LVEF was $>50\%$ in most patients, and LVEF did not increase in the control or the EPO groups. (D) r-EF increased significantly in the EPO group ($P=0.0414$), but not in the control group ($P=0.1122$). (E) In the LAD group r-EF increased significantly in the EPO group ($P=0.0137$), but not in the control group ($P=0.7344$). (F) In the non-LAD r-EF decreased significantly in the control group ($P=0.156$), but not in the EPO group ($P=0.7422$). Open circles and bars, mean and SD, respectively.

2 groups (control, $+4.1\pm12.1\%$; EPO, $+7.6\pm12.5\%$, $P=0.4737$). r-EF also increased significantly in the EPO group (from $40.3\pm20.6\%$ to $46.1\pm20.5\%$, $P=0.0414$), but not in the control group (from $41.1\pm20.8\%$ to $34.6\pm13.4\%$, $P=0.1121$), and there was a significant difference of Δ r-EF between the 2 groups (control, $-6.5\pm13.3\%$; EPO, $+5.8\pm13.5\%$, $P=0.0094$). Changes in the EDVI were not significant between the control and EPO groups. ESVI decreased significantly in the EPO group (from $49.0\pm34.2\%$ to $36.5\pm22.4\%$, $P=0.0192$), but not in the control group (from $53.7\pm27.6\%$ to $45.8\pm27.2\%$, $P=$

0.0512). There was no significant difference of Δ ESVI between the 2 groups (control, $-7.9\pm14.5\%$; EPO, $-12.5\pm17.9\%$, $P=0.6444$). Infarct size decreased significantly in the control (from $31.5\pm18.2\%$ to $21.3\pm16.1\%$, $P=0.0006$) and EPO groups (from $29.8\pm18.5\%$ to $17.8\pm15.3\%$, $P=0.0008$), but Δ Infarct size was almost the same in the control (-10.2 ± 10.6) and EPO groups (-12.0 ± 12.4). Regional uptake from baseline to 6-month follow up was not significant between the control and EPO groups.

As shown in Figure 2, LVEF was $<50\%$ in most patients

Table 4. ^{99m}Tc-MIBI Scintigraphy of Left Ventricular Volume, Infarct Size, and Regional Uptake vs Time

		Baseline	6-month follow up	Control	EPO	P value
Grobal LVEF (%)						
All	Control	47.2±16.2	51.3±18.2			NS
	EPO	51.0±19.6	58.5±15.0			0.0238
LAD	Control	35.3±8.1	40.8±14.9			NS
	EPO	37.5±13.0	52.7±15.8			0.0049
Non-LAD	Control	62.4±9.3	64.9±12.5			NS
	EPO	67.4±12.1	65.6±11.0			NS
r-EF (%)						
All	Control	41.1±20.8	34.6±13.4			NS
	EPO	40.3±20.6	46.1±20.5			0.0414
LAD	Control	30.8±14.1	31.3±8.4			NS
	EPO	37.6±17.1	47.2±20.5			0.0137
Non-LAD	Control	54.5±21.1	38.8±17.8			0.0156
	EPO	44.1±25.4	44.7±21.8			NS
Left ventricular EDVI (ml/m²)						
All	Control	96.5±27.5	85.5±27.8			NS
	EPO	90.8±41.0	82.3±31.1			NS
LAD	Control	112.1±25.3	95.9±26.2			NS
	EPO	112.0±42.3	92.5±37.4			0.0049
Non-LAD	Control	76.4±14.1	72.1±25.5			NS
	EPO	64.9±19.7	69.8±15.2			NS
Left ventricular ESVI (ml/m²)						
All	Control	53.7±27.6	45.8±27.2			NS
	EPO	49.0±34.2	36.5±22.4			0.0192
LAD	Control	72.8±20.5	59.1±27.0			0.0391
	EPO	71.3±29.7	46.1±24.7			0.0010
Non-LAD	Control	29.1±10.1	28.7±16.3			NS
	EPO	21.6±12.3	24.7±12.2			NS
Infarct size (%)						
All	Control	31.5±18.2	21.3±16.1			0.0006
	EPO	29.8±18.5	17.8±15.3			0.0008
LAD	Control	43.1±12.9	28.1±16.6			0.0039
	EPO	40.9±14.7	23.2±17.5			0.0010
Non-LAD	Control	16.7±12.2	12.5±11.1			0.0312
	EPO	14.6±10.9	10.4±7.8			NS
Regional uptake (%)						
All	Control	56.5±11.1	64.6±11.5			NS
	EPO	58.0±12.3	65.4±11.5			NS
LAD	Control	54.4±9.3	62.8±9.7			NS
	EPO	55.9±14.5	65.4±14.5			NS
Non-LAD	Control	59.1±13.4	66.9±13.9			NS
	EPO	60.9±8.5	65.4±6.6			NS
ΔLVEF (%)						
All				+4.1±12.1	+7.6±12.5	NS
LAD				+5.4±14.8	+15.3±9.4	NS
Non-LAD				+2.4±8.4	-1.9±8.9	NS
Δr-EF (%)						
All				-6.5±13.3	+5.8±13.5	0.0094
LAD				+0.6±10.0	+9.6±9.2	NS
Non-LAD				-15.6±11.7	+0.6±17.2	0.0401
ΔEDVI (ml/m²)						
All				-11.0±21.5	-8.5±20.0	NS
LAD				-16.3±23.5	-19.5±17.2	NS
Non-LAD				-4.2±17.9	+4.9±14.6	NS
ΔESVI (ml/m²)						
All				-7.9±14.5	-12.5±17.9	NS
LAD				-13.7±15.4	-25.2±13.5	NS
Non-LAD				-0.4±10.0	+3.1±5.8	NS
ΔInfarct size (%)						
All				10.2±10.6	12.0±12.4	NS
LAD				15.0±12.2	17.7±12.2	NS
Non-LAD				4.1±2.6	4.1±7.9	NS
ΔRegional uptake (%)						
All				8.1±3.5	7.0±3.3	NS
LAD				8.3±3.8	9.5±4.8	NS
Non-LAD				7.7±7.9	4.5±5.4	NS

Data are Mean±SD.

LVEF, left ventricular ejection fraction; non-LAD, left circumflex artery or right coronary artery; r-EF, regional LVEF; EDVI, end-diastolic volume index; ESVI, end-systolic volume index. Other abbreviations see in Table 1.

Table 5. Plasma BNP Level (pg/ml)

	Control	EPO
Day 14		
All	163.1±120.5 (18)	133.1±84.0 (20)
LAD	169.9±124.6 (10)	145.0±91.3 (11)
Non-LAD	154.6±123.0 (8)	118.6±76.8 (9)
6 months		
All	72.3±125.9 (15)	53.0±34.2 (16)
LAD	35.5±25.4 (8)	48.2±27.5 (10)
Non-LAD	114.3±180.0 (7)	61.0±44.9 (6)
ΔBNP		
All	-80.3±106.0 (15)	-84.3±97.8 (15)
LAD	-120.3±106.5 (8)	-80.6±111.1 (10)
Non-LAD	-34.7±91.7 (7)	-91.9±74.9 (5)

Data are mean±SD (n). Normal range: <18.4 pg/ml. There were no significant differences among the 2 groups. BNP, brain natriuretic peptide. Other abbreviations see in Table 1.

in the LAD subgroup in the early post-MI phase, while it was >50% in most patients in the non-LAD subgroup. In the LAD subgroup, LVEF increased significantly in the EPO group (from 37.5±13.0% to 52.7±15.8%, $P=0.0049$), but not in the control group (from 35.3±8.1% to 40.8±14.9%, $P=0.3594$). In the non-LAD subgroup, LVEF did not increase either in the EPO group (from 67.4±12.1% to 65.6±11.0%, $P=0.4609$) or in the control group (from 62.4±9.3% to 64.9±12.5%, $P=0.5625$). In the LAD subgroup, r-EF increased significantly in the EPO group (37.6±17.1% to 47.2±20.5%, $P=0.0137$), but not in the control group (30.8±14.1% to 31.3±8.4%, $P=0.7344$). In the non-LAD subgroup, r-EF decreased significantly in the control group (from 54.5±21.1% to 38.8±17.8%, $P=0.0156$), but not in the EPO group (from 44.1±25.4 to 44.7±21.8%, $P=0.7422$). There was a significant difference of Δr-EF in the non-LAD subgroup (control, -15.6±11.7; EPO, +0.6±17.2; $P=0.0401$), but not in the LAD subgroup (control, +0.6±10.0; EPO, +9.6±9.2; $P=0.0947$). No significant differences of ΔEDVI, ΔESVI or ΔInfarct size were observed in the LAD or non-LAD subgroups.

Plasma BNP Level and Cardiac Catheterization

There was no significant difference of plasma BNP levels in the control and EPO groups, as shown in Table 5. During 6-month follow-up catheterization of the 36 patients, LVG and CAG were performed in 31 subjects (control, 14; EPO, 17) and 35 subjects (control, 16; EPO, 19), respectively. On LVG, improvement in LVEF was observed in the EPO group (baseline, 53.9±11.6; 6-month follow up, 60.6±9.7, $P=0.0103$), but not in the control group (baseline, 57.2±9.4; 6-month follow up, 59.0±15.0, $P=0.6698$). On CAG, no significant difference ($P=0.8990$) was observed in the frequency of in-stent restenosis in the 6-month follow-up period between the control (4/16, 25.0%) and EPO groups (4/19, 21.1%). No significant difference in MLD or percent diameter stenosis was observed between the 2 groups on QCA (Table 6).

Adverse Clinical Events

After randomization 1 sudden death in the control group (non-LAD, baseline LVEF 49%), 1 subacute stent thrombosis in the control group, 1 vasospastic angina in the control group, 1 leg edema in the EPO group, 1 rhabdomyolysis due to statin in the EPO group, and 1 leukocytopenia due to thienopyridine in the EPO group were observed. There were no

Table 6. Quantitative Coronary Angiography vs Holter ECG

	Control	EPO
Before PCI		
RD (mm)	2.89±0.44	2.96±0.55
MLD (mm)	0.49±0.18	0.49±0.14
%DS	97.9±6.4 (16)	97.2±8.4 (20)
After PCI		
RD (mm)	2.95±0.54	3.15±0.48
MLD (mm)	2.64±0.58	2.68±0.51
%DS	10.1±14.9	15.4±11.7
Acute gain	2.59±0.63 (16)	2.63±0.58 (20)
6-month follow up		
RD (mm)	2.86±0.57	2.83±0.43
MLD (mm)	2.02±0.59	1.98±0.72
%DS	28.2±18.4	29.5±24.3
Late loss	0.60±0.53 (15)	0.70±0.71 (20)
Before PCI		
VPC (beats/day)	124.7±296.9 (14)	8.2±12.2 (17)
6-month follow up		
VPC (beats/day)	269.2±443.0 (14)	119.0±273.0 (17)

Data are mean±SD (n). There were no significant differences among the 2 groups.

ECG, electrocardiogram; RD, reference diameter; MLD, minimal lumen diameter; %DS, diameter stenosis %; VPC, ventricular premature complexes. Other abbreviations see in Table 1.

critical adverse events (MACE or thrombosis) in the EPO group.

There were no differences between the control and EPO groups concerning the number of premature ventricular complexes per day (Table 6) or the occurrence of non-sustained or sustained ventricular tachycardias on Holter monitoring at baseline and at 6-month follow up.

Discussion

In the present study EPO administration significantly improved LVEF, and the improvement of LVEF was as much as 15.3% (control: 5.4%) in the LAD subgroup despite the fact that all the patients had undergone successful PCI. Although the reason is unknown, r-EF deteriorated in the non-LAD subgroup, and EPO administration prevented the change. No significant difference, however, was observed in the direct comparisons of the control and EPO groups in LVEF, EDVI, ESVI, or infarct size, presumably because of the small number of cases as shown in Table 4. Therefore, a larger scale study is necessary to produce concrete evidence of the efficacy of EPO administration in patients with AMI.

So far, several adjuvant therapies for PCI have been undertaken to treat patients with AMI, such as granulocyte colony-stimulating factor administration¹⁸ and intracoronary stem cell administration,¹⁹ and the results have been controversial. Administration of EPO and 1 of its analogues have also been tried. A single bolus of darbepoietin (300 mg), a long-acting analog of EPO, was safely administered, but showed no efficacy.¹⁴ Another trial of a single bolus with 60,000 IU of EPO (HEBE-III) is ongoing.¹⁵ Administration of 33,333 IU rhEPO once a day for 3 consecutive days, REVIVAL-3, was not effective and called attention to the possible risk of EPO administration leading to major cerebral and cardiovascular events in preliminary analysis.

Thrombopoietin (TPO) induces thrombopoiesis and primes

platelet aggregation. EPO has weak TPO-like activity, and sometimes induces thrombocytosis and platelet activation that increases the risk of thrombosis and cardiovascular events as a result.²⁰ Therefore, a high dose of EPO may induce thrombosis in the injured coronary arteries after PCI. A single dose of 12,000 IU rhEPO has been widely used for dialysis patients safely, and a single dose may be enough to protect cardiomyocytes from apoptosis. In the present study we adopted a single medium dose of EPO to avoid possible promotion of vascular events in the brain and heart. We also administered continuous heparin as well as at least 2 different anti-platelets for the prevention of vascular events. A single dose of 12,000 IU EPO does not promote polycythemia in humans while maintaining serum EPO level as high as 1 IU/ml throughout the 24 h after EPO administration, and that might be a sufficient concentration to protect the myocardium from ischemia–reperfusion injury. Concerning the response to anemia or hypoxia, sustained elevation of EPO levels occurs through its production in the kidney. In fact, a pharmacokinetic study of rhEPO in healthy human volunteers showed that multiple administrations of EPO to maintain the EPO level in vivo was necessary for the activation of erythropoiesis, and EC50 (concentrations to stimulate up to 50% of the maximal effect) was 27–61 mIU/ml.²¹ In contrast, a higher level of EPO is required for tissue protection in the very early phase of injury.²² Interestingly, rhEPO protects the myocardium from ischemia–reperfusion injury and promotes beneficial remodeling, and the improvement of cardiac function is unrelated to infarct size.²³ Therefore, it is possible that short-term elevation of EPO level is sufficient for improvement of cardiac function in an infarct size-independent manner that does not stimulate erythropoiesis in vivo.

In the present clinical study, serum EPO levels significantly increased within 24 h after PCI in the control group. In a previous report, a Hb-independent increase of EPO was also observed in patients with AMI, which might indicate the intrinsic auto/paracrine secretion of EPO in vivo,²⁴ while the serum EPO level rose in an Hb-dependent manner in chronic heart failure.²⁵ In a study of patients with AMI subjected to successful primary PCI, a higher endogenous EPO level (>20.3 mU/ml) predicted a smaller infarct size that might be attributed to the potentially protective effect of endogenous EPO against ischemia–reperfusion injury in humans.²⁶ The circulating EPO level is also higher (>23 mIU/ml) in patients with congestive heart failure, and the level is a strong predictor of mortality.²⁷ Thus exogenous EPO administration may enhance the insufficient cardioprotection system of intrinsic EPO.

The present EPO/AMI-1 pilot study is the first report to show the safety and efficacy of a single dose of EPO to treat patients with AMI. A further large-scale clinical trial is necessary to show the striking effects of EPO on AMI with reliable evidence. We therefore have decided to conduct a large double-blind study: the EPO/AMI-2 trial. In the following study, 250 patients with an infarct-related artery in LAD will be enrolled.

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Appendix 1

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