**Background:** Erythropoietin (EPO) has antiapoptotic and tissue-protective effects, but previous clinical studies using high-dose EPO have not shown cardioprotective effects, probably because of platelet activation and a lack of knowledge regarding the optimal dose. In contrast, a small pilot study using low-dose EPO has shown improvement in left ventricular function without adverse cardiovascular events.

**Methods and Results:** We performed a multicenter (25 hospitals), prospective, randomized, double-blind, placebo-controlled, dose-finding study to clarify the efficacy and safety of low-dose EPO in patients with ST-segment elevation myocardial infarction (STEMI) under the Evaluation System of Investigational Medical Care of the Ministry of Health, Labor and Welfare of Japan. In total, 198 STEMI patients with low left ventricular ejection fraction (LVEF <50%) were randomly assigned to receive intravenous administration of EPO (6,000 or 12,000 IU) or placebo within 6 h of successful percutaneous coronary intervention. At 6 months, there was no significant dose-response relationship in LVEF improvement among the 3 groups tested (EPO 12,000 IU: 5.4±9.3%, EPO 6,000 IU: 7.3±7.7%, Placebo: 8.1±8.3%, P=0.862). Low-dose EPO also did not improve cardiac function, as evaluated by \(^{99m}\)Tc-MIBI SPECT or NT-proBNP at 6 months and did not increase adverse events.

**Conclusions:** Administration of low-dose EPO did not improve LVEF at 6 months in STEMI patients (UMIN000005721).

**Key Words:** Acute myocardial infarction; Erythropoietin; Left ventricular ejection fraction
remodeling and enhances neovascularization.\(^6\)\(^-\)\(^8\) Several clinical studies have been performed to clarify the cardioprotective effects of EPO in patients with AMI. However, administration of high-dose EPO (60,000–99,000 IU) has not been found to improve LV ejection fraction (LVEF) or to decrease infarct size.\(^9\)\(^-\)\(^11\) Platelet activation by high-dose EPO\(^12\) and the lack of a known optimal dose of EPO for limiting infarct size may have resulted in these negative outcomes.\(^13\) In contrast, in the EPO-AMI-I study, a multicenter, prospective, randomized, single-blind study, performed to clarify the effects of the low-dose (12,000 IU) of EPO on the improvement of LVEF at 6 months in 36 patients with ST-segment elevation MI (STEMI),\(^14\) low-dose EPO was associated with improved LV function without major cardiovascular events (MCE). Furthermore, a retrospective analysis of EPO-AMI-I revealed that administration of low-dose EPO was highly associated with improved LV function in patients with low LVEF (<50%).\(^14\) Therefore, we performed EPO-AMI-II, a prospective, randomized, double-blind, placebo-controlled, dose-finding study (6,000 IU and 12,000 IU), multicenter study in first STEMI patients to clarify the efficacy and safety of low-dose EPO in STEMI patients with low LVEF (<50%).

**Methods**

**Study Design**

The protocol for EPO-AMI-II was approved by both the Evaluation System of Investigational Medical Care of the Ministry of Health, Labor and Welfare of Japan and the Japanese governmental health insurance system. It was also approved by the ethics committees of the participating hospitals and the study was performed in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained before the enrollment of all patients. The study was registered at the UMIN Clinical Trials Registry as UMIN000005721.

**Patients**

We prospectively enrolled patients who were ≥20 and ≤80 years of age, with a first STEMI and successful reperfusion by PCI within 12 h of symptom onset. According to a subanalysis of EPO-AMI-I, patients with low LVEF (<50% at enrollment, measured by echocardiography or left ventriculography) were enrolled because they were likely to benefit most from low-dose EPO.\(^14\) Exclusion criteria included residual significant stenosis requiring revascularization, impaired reperfusion (TIMI flow grade ≤1), Killip class III or IV or cardiogenic shock at admission, advanced renal or hepatic dysfunction (creatinine ≥2 mg/dL or total bilirubin ≥3 mg/dL), blood pressure ≥140/90 mmHg after PCI, hematocrit ≥54% at admission, atrial fibrillation after PCI, malignant hypertension, previous EPO administration, blood transfusion in the previous 3 months, cancer in the previous 5 years, severe infection, contraindications for aspirin or thienopyridine derivatives, pregnancy and breastfeeding.

**Study Protocol**

Standard antiplatelet treatments for AMI were administered prior to or at the time of primary PCI. The enrolled patients were randomly divided into 3 groups (epoetin-β 12,000 or 6,000 IU) or placebo. EPO or placebo was diluted in 10 mL of saline and administered intravenously over the course of 1 min within 6 h of PCI. The double-blind administration was ensured by a subject identification code unknown to the physicians, nurses or patients. Drug or placebo was prepared under medical supervision according to instructions contained in predefined packages provided by the EPO-AMI-II organization. Standard treatments, including β-blockade, lipid-lowering therapy, and angiotensin-converting enzyme inhibition or angiotensin-II receptor blockade, were additionally prescribed. Patients were followed for 6 months after enrollment. EPO and placebo were provided by Chugai Pharmaceutical Co. Ltd. (Tokyo, Japan).

**Endpoints**

The primary endpoint was LVEF improvement between the acute (4–7 days) and chronic stages (6 months). The secondary endpoints were the efficacy and safety of EPO treatment. Efficacy was evaluated on the basis of indexes of cardiac function measured by \(^99\)mTc-MIBI SPECT, such as LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESD), LVEDV index, LVESV index, summed motion score (SMS), uptake at rest, summed rest score (SRS), summed difference score (SDS), defect size at rest, and defect size with stress and ischemia. The survival rate, MCE (defined as cardiac death, stroke, non-fatal MI, admission for worsening of HF, admission for unstable angina (UAP), revascularization, and onset of HF symptoms) and NT-proBNP at 6 months were also analyzed to evaluate efficacy. Safety was evaluated on the basis of the incidence of adverse events, clinical laboratory tests and vital signs.

**Quantification of LV Function and Infarct Size**

We performed ECG-gated \(^99\)mTc-MIBI SPECT (600–740 MBq, FUJIFILM RI Pharma Co., Ltd, Tokyo, Japan) 4–7 days and 6 months after administration of EPO or placebo. ECG-gated SPECT image acquisition was performed 60 min after \(^99\)mTc-MIBI injection. With ECG gating, the SPECT data divided into 16 equal intervals were analyzed by Quantitative Gated SPECT software (Cedars-Sinai Medical Center, Los Angeles, CA, USA).\(^15\) A small heart, defined on the basis of an end-systolic volume <20 mL, was excluded from the analysis of RF, LVESV and LVESVI because underestimation of LVESV and overestimation of EF would probably be caused by inappropriate delineation of the left ventricle.\(^16\)

Adenosine stress tests were also performed at 6 months with non-gated \(^99\)mTc-MIBI SPECT. Adenosine (Adenoscan; Daiichi Sankyo, Tokyo, Japan) was administered at a rate of 0.72 mg/kg for 6 min. The \(^99\)mTc-MIBI was injected 3 min after the start of adenosine infusion. Uptake and defect size were calculated by Quantitative Perfusion SPECT software (Cedars-Sinai Medical Center).\(^17\) Uptake was assessed by application of a 17-segment model of the left ventricle according to the standard myocardial segmentation of the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association and was expressed as the mean uptake count in these segments.\(^18\) Defect size was expressed as the percentage of defects at less than the threshold of 60% of peak counts in the entire left ventricle. SPECT data were analyzed in a blinded fashion with the assistance of nuclear medicine special radiological technologists at MICRON Co., Ltd. (Tokyo, Japan) and the Japan Academic Research Forum (Osaka, Japan). Finally, the analyzed data were evaluated by an independent radioisotope (RI) assessment committee.
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Clinical and Laboratory Measures
Blood pressure and heart rate were monitored at regular intervals until patient discharge and were recorded at baseline, 1h, 4, 7 and 35 days and 6 months after administration of EPO or placebo. Hematologic tests were measured at baseline and 4, 7 and 35 days and 6 months. Reticulocytes were measured at baseline and 7 days, because we had confirmed in EPO-AMI-I that they peaked 7 days after EPO administration and decreased thereafter. NT-proBNP was measured at 6 months. Adverse events were recorded during hospitalization and up to 6 months thereafter.

Sample Size Calculation and Interim Analysis
According to the results of EPO-AMI-I, we estimated the difference in LVEF improvement between the EPO (12,000 IU) and placebo group to be 4.42%, the common standard deviation to be 14.33%, and the effect size to be 0.31. To demonstrate a treatment difference with a power of 0.85 and a 1-sided type 1 error of 0.025, 190 patients per group were required. By taking into account 2 interim analyses and several patients dropping out, the recruited sample size was determined to be 200 patients per group (i.e., 600 patients in total).

We designed 2 formal interim analyses for the primary endpoint and safety: when 198 and 396 enrolled patients, respectively, completed 6-months of follow-up. An independent data safety monitoring board (DSMB) evaluated the primary endpoint according to the rules determined by the Lan-DeMets method with the O’Brien-Fleming error spending function. Asymmetric stopping boundaries were planned, in which early termination of the study was to be recommended with evidence of either overwhelming benefit (1-sided favoring EPO) or futility (1-sided against EPO). There was no statistical rule used for terminating this study because of safety concerns. The DSMB recommendations were based on the clinical assessment of the frequency of severe adverse events, their nature and relation to the investigational treatment.

Statistical Analysis
Summary statistics of quantitative continuous variables are presented as the mean ± standard deviation (SD). NT-proBNP is presented as both mean ± SD and median (interquartile range [IQR]), owing to heteroscedasticity. Categorical data are presented as absolute frequencies. Data were analyzed on the basis of the intention-to-treat principle. For LVEF improvement, the null hypothesis that all treatment groups had the same mean LVEF improvement was tested against the alternative hypothesis that the mean LVEF improvement increased in the order of placebo, EPO (6,000 IU) and EPO (12,000 IU). This test was performed by the linear contrast test with coefficient (−1, 0, 1) and the t-statistic. The primary endpoint was further analyzed within the subgroups of the location of...
culprit lesion (left anterior descending coronary artery (LAD) vs. non-LAD) and sex. All other $^{99m}$Tc-MIBI SPECT data were compared by Jonckheere-Terpstra test. Cardiovascular events and NT-proBNP were also compared by Jonckheere-Terpstra test. The proportion of patients with ischemia was compared by the Cochran-Armitage test. Survival rates were analyzed by the Kaplan-Meier method and compared by log-rank test. Reticulocyte counts were compared by one-way ANOVA. All data were evaluated by a linear contrast test based on a 1-sided significance level of 0.025, except for major cardiovascular or adverse events, which were evaluated on the basis of a 2-sided significance level of 0.05.
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Results

First Interim Analysis
From December 2011 to February 2015, a total of 198 patients were enrolled at 25 hospitals. After the 6-month follow-up of the final randomized patient, the first interim analysis was performed. The DSMB recommended study termination because the results of the analysis met the futility stop criterion. Therefore, the principal investigators decided to terminate the study and performed the final analysis.

Patients’ Enrollment and Baseline Characteristics
Of the 198 patients, 68 were assigned to the EPO 12,000 IU group, 70 to the EPO 6,000 IU group and 60 to the placebo group (Figure 1). One patient (EPO 6,000 IU) was excluded from the safety evaluation because the study drug was not administered within 6h after PCI; 10 patients were excluded from the efficacy evaluation because 7 (EPO 12,000 IU: 4, EPO 6,000 IU: 1, Placebo: 1) withdrew their consent, and 1 (EPO 6,000) died of non-cardiac cause. Additionally, 47 patients were excluded from the primary endpoint evaluation because 14 (EPO 12,000 IU: 4, EPO 6,000 IU: 6, Placebo: 4) had small hearts, 11 (EPO 12,000 IU: 4, EPO 6,000 IU: 1, Placebo: 2) had poor image quality, 18 (EPO 12,000 IU: 4, EPO 6,000 IU: 1, Placebo: 2) deviated from the schedule, 2 (EPO 12,000 IU: 1, Placebo: 1) were mistakenly administered thallium instead of technetium and 2 (EPO 6,000 IU: 1, Placebo: 1) had adverse events. The baseline characteristics are shown in Table 1.

Improvement of LVEF
At 6 months, the mean (±SD) LVEF increased in all groups from baseline (EPO 12,000 IU: from 45.1±11.3% to 50.7±11.4%, EPO 6,000 IU: from 47.0±12.2% to 53.4±10.5%; Placebo: from 45.4±11.5% to 52.3±10.8%), as shown in Figure 2. However, there was no significant dose–response relationship in the LVEF improvement at 6 months (ΔLVEF) among the 3 groups (EPO 12,000 IU: 5.4±9.3%; EPO 6,000 IU: 7.3±7.7%; Placebo: 8.1±8.3%, P=0.862) (Table 2). We also found that ΔLVEF did not differ between patients with LAD culprit lesions (n=156) and those with non-LAD ones (n=41) (LAD: 7.3% vs. non-LAD 5.9%, P=0.56). Furthermore, we found that ΔLVEF in men (n=169) was significantly superior to that in women (n=28) (men: 7.2% vs. women 4.1%, P=0.02).

Efficacy of EPO Towards Cardiac Function and Ischemia by 99mTc-MIBI SPECT
There was no significant dose–response relationship in the differences between 4–7-day and 6-month evaluations for LVEDV, LVESV, LVEDVI, LVESVI, SMS, uptake at rest, SRS and defect size at rest among the 3 groups (Table 3). There was also no significant dose-response relationship in SDS, defect size with stress and ischemia at 6 months.

Plasma NT-proBNP Level
There was no significant dose-response relationship in plasma NT-proBNP levels at 6 months (EPO 12,000 IU: 201 pg/mL [110–391]; EPO 6,000 IU: 224 pg/mL [96–399]; Placebo: 144 pg/mL [102–254], P=0.909) (Table 4).

Survival Rate
There were no cardiac deaths, and 1 non-cardiac death occurred in the EPO 6,000 IU group. There were no
significant differences in survival rate among the 3 groups (P=0.526) (Figure 3).

**Major Cardiovascular Events and Adverse Events**

Major cardiovascular events and adverse events during the 6 months after enrollment are shown in Table 5 and Table S1, respectively. For MCE, there were no significant differences among the 3 groups (EPO 12,000 IU: 4 patients (5.9%) [1 stroke, 1 admission for worsening of HF, 1 admission for UAP and 1 onset of HF symptoms]; EPO 6,000 IU: 4 patients (5.8%) [1 stroke, 1 non-fatal MI, 2 admissions for UAP and 2 onset of HF symptoms]; Placebo:...
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4 patients (6.7%) [1 admission for worsening of HF, 1 for revascularization and 2 for onset of HF symptoms], P=0.877). Additionally, there were also no significant differences in the incidence non-cardiac death, intracardiac thrombi, endomyocarditis, Dressler syndrome, ventricular tachycardia, atrial fibrillation/flutter and sick sinus syndrome. No other venous embolic and thrombotic events were confirmed during the 6 months after EPO administration among groups.

Laboratory Tests and Vital Signs

Reticulocyte counts tended to increase from baseline to 7 days after EPO administration (EPO 12,000 IU: 25.3±30.6×10^3 cells/μL; EPO 6,000 IU: 20.5±25.1×10^3 cells/μL; Placebo: 9.8±29.0×10^3 cells/μL, P=0.032) (Table S2A). There were no increases with EPO administration in red blood cell numbers, hemoglobin, hematocrit, platelets, systolic or diastolic pressure or heart rate (Tables S2B, S3).

Discussion

In STEMI patients with low LVEF (<50%), a single administration of low-dose EPO (epoetin-β 12,000 or 6,000 IU) within 6 h after successful PCI did not improve LVEF, cardiac function or NT-proBNP at 6 months. However, administration of low-dose EPO did not increase MCE.

Previous proof-of-concept studies using high-dose EPO have reported negative results in patients with STEMI.14–16 In contrast, low-dose EPO was deemed likely to be cardioprotective in a small clinical study (EPO-AMI-I).14 Potential mechanisms explaining the dose-dependent discrepancy of EPO in cardioprotection might be attributable to platelet activation and the existence of an unknown lower optimal dose for limiting infarct size.12,13 Because EPO has structural similarity to thrombopoietin,12 high doses increase platelet production and reactivity, thus leading to an increased risk of thrombosis and cardiovascular events. Additionally, the dose-response curve analyzing the bioactivity of cytokines does not appear to follow a sigmoid function. Positive intracellular signals of cytokine receptors, mediated by serial chain reactions of protein tyrosine kinases, are typically interfered with by automatic circuit reactions of protein tyrosine phosphatases, such as SHP1, to avoid overstimulation of growth and inflammation.19 Thus, high-dose EPO might have less cardioprotective activity in rat and mouse coronary ischemia/reperfusion models.

Contrary to our speculation, beneficial efficacy of low-dose EPO was not observed in EPO-AMI-II. There was no dose-response relationship in LVEF improvement among the 3 groups, and the LVEF improvement in the placebo group was somewhat higher than that in the EPO 12,000 and 6,000 IU treatment groups. Additionally, there was no dose-response relationship in cardiac function, as evaluated by 99m Tc-MIBI SPECT and NT-proBNP. Because reperfusion injury starts in the very early phase of recanalization of an infarct-related artery, drugs to prevent reperfusion injury should be administered as soon as possible after reperfusion.20 Although the administration of EPO within 24 h after successful PCI was effective for cardioprotection in EPO-AMI-I,14 this late administration of EPO would have contributed to the negative results in EPO-AMI-II. Additionally, patients with low LVEF (<50%) were enrolled in the present study according to a subanalysis of EPO-AMI-I. In reality, the culprit lesion was most often located in the LAD, and the severity of AMI was sufficient to evaluate the efficacy of low-dose EPO. However, we cannot deny the possibility that other confounders, such as

<table>
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<th>Table 5. Major Cardiovascular Events During 6 Months in EPO-AMI-II Study</th>
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<td>Major cardiovascular events</td>
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<td>Cardiac death</td>
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<td>Stroke</td>
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<td>Non-fatal MI</td>
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<td>Admission for worsening of HF</td>
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<td>Admission for UAP</td>
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<td>Revascularization</td>
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<td>Onset of HF symptoms</td>
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Data are presented as n (%). MI, myocardial infarction; UAP, unstable angina pectoris. Other abbreviations as in Table 1.
coronary collateralization to the area at risk, that were not controlled for in EPO-AMI-II might have contributed to the different results from those in EPO-AMI-I.  

The primary endpoint was further analyzed within the subgroups of the location of the culprit lesion (LAD vs. non-LAD) and sex. Although LV improvement did not differ between patients with LAD culprit lesions (n=156) and those with non-LAD ones (n=41), we found that LV improvement in men (n=169) was significantly superior to that in women (n=28). We need caution when we consider these results, because the numbers in the subgroups were very different.

Increases in major cardiovascular events and adverse events were not observed in this study or in EPO-AMI-I. Although we did not perform special examination to determine venous embolic and thrombotic events, none were confirmed during the 6 months after EPO administration. Although we did not perform special examination to determine venous embolic and thrombotic events, none were confirmed during the 6 months after EPO administration.

In contrast, in the HEBE III study, subanalysis revealed that EPO treatment showed a trend towards a decrease in enzymatic infarct size, and the incidence of the combined endpoint (cardiovascular death, MI, in-stent thrombosis, UAP and HF) was significantly decreased. In the REVIVAL study, EPO (33,000 IU×3) treatment showed a trend towards an increased rate of severe adverse events. A meta-analysis showed that the administration of EPO appeared to be safe for patients with acute STEMI. In the present study, reticulocyte counts tended to increase from baseline to 7 days with EPO administration, owing to hematopoietic effects. However, there were no differences in red blood cell numbers, hemoglobin, hematocrit, platelets or vital signs. These results indicated that administration of low-dose EPO in STEMI patients was safe.

Study Limitations
This study was terminated at the first interim analysis, so it was not powered for clinical events, and the previous statements should be carefully interpreted. However, it is unlikely that positive findings were missed because of insufficient power.

Conclusions
In STEMI patients with low LVEF (<50%), we compared the effect of intravenous administration of low-dose EPO (12,000 or 6,000 IU) or placebo within 6h after successful PCI. Low-dose EPO did not increase LVEF improvement 6 months after AMI.

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Funding Sources

References
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Supplementary Files

Supplementary File 1
Table S1. Adverse events during 6 months
Table S2. (A) Reticulocyte counts, (B) laboratory tests
Table S3. Vital signs
Please find supplementary file(s): http://dx.doi.org/10.1253/circj.CJ-17-0889