Erythropoietin in cardiac ischemia

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Chapter 6

A single bolus of a long-acting erythropoietin analogue darbepoetin alfa in patients with acute myocardial infarction: a randomized feasibility and safety study

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Abstract

Aims: Besides stimulating hematopoiesis, erythropoietin (EPO) protects against experimental ischemic injury in the heart. The present study evaluated the safety and tolerability of EPO treatment in non-anemic patients with acute myocardial infarction (MI).

Methods and Results: In this single-center, investigator-initiated, prospective study, patients with a first acute MI were randomized to one bolus of 300 μg darbepoetin alfa or no additional medication before primary coronary intervention. Twenty-two patients (mean age 59±2 years) were included. In the darbepoetin group, serum EPO-levels increased to 130-270 times that of controls, within the first 24-hours. After darbepoetin administration, only small and non-significant changes in hematocrit levels were observed, while endothelial progenitor cells (EPCs, CD34+/CD45-) were increased at 72-hours (2.8 vs. 1.0 cells/μl in control group; $p<0.01$). No adverse events were recorded during the 30-day follow-up. After 4 months, left ventricular ejection fraction was similar in the two groups (52±3% in darbepoetin vs. 48±5% in control group, p=NS).

Conclusions: Intravenous single high-dose darbepoetin alfa in acute MI is both safe and well tolerated. Darbepoetin treatment after MI stimulates EPCs mobilization. The results of this first pilot study support a larger scale clinical trial to establish efficacy of EPO administration in patients after acute MI.

Introduction

Erythropoietin (EPO) is a glycoprotein produced primarily by the kidney in response to hypoxia and subsequently targets erythroid progenitor cells in bone marrow to increase the number of erythrocytes ([1]).

Independent of its hematopoietic effect, EPO was recently shown to be protective against ischemic injury in various organs, including the heart. A functional EPO receptor was found in rat myocardium, expressed mainly on endothelial cells ([2]). These results were recently expanded by a finding of EPO receptor expression in human heart tissue. Both ventricular myocytes and endothelial cells in the adult human heart were positive for the EPO receptor ([3]). In several experimental studies, administration of EPO both during cardiac ischemia-reperfusion or after permanent coronary artery occlusion, led to infarct size reduction and improvement in left ventricular (LV) function ([4,5]). Clinically important, our group showed the extension of the EPO cardioprotective effect when applied after the onset of ischemia and even after the start of reperfusion ([6]). Protection against apoptosis was implicated as a possible mechanism of the observed EPO effects ([5,7]).

In addition to its anti-apoptotic effects, EPO was shown to stimulate the mobilization of endothelial progenitor cells (EPCs) in bone marrow ([8]). Mobilization and activation of these progenitor cells was associated with neovascularization of ischemic myocardium, a process that may play a beneficial role in the later phase of post-myocardial infarction (MI) LV remodeling ([9]).

Epoetin alfa is the recombinant (rhEPO) form of the endogenous cytokine, with equal biological activity ([10]). RhEPO has been used in clinical settings for more than a decade, initially for the management of anemia caused by end stage renal disease ([11]). The indication for EPO treatment has broadened in the last few years also to include other forms of anemia

Table 1. Inclusion and exclusion criteria

Inclusion criteria
Male between 18-76
Acute myocardial infarction diagnosed by:
- chestpain suggestive for acute myocardial infarction
- symptom onset ‹ 6 hours
- ECG with ST-T elevation › 1 mV in 2 or more leads
Indication for primary angioplasty
Killip class I
Exclusion criteria
Hemoglobin levels › 16.0 g/dL
Acute myocardial infarction pre-treated with thrombolysis
Previous myocardial infarction
Systolic bloodpressure › 150 mmHg
Diastolic bloodpressure › 95 mmHg
Serum creatinine › 140 µmol/l
Previous treatment with rh-EPO
Blood transfusion ‹ 12 weeks prior to randomization
Indication for blood transfusion
Polycythemia vera
Concomitant inflammatory or malignant disease
Recent trauma or major surgery
Previous thrombo-embolic complication

where EPO deficiency is not causative (myelodysplastic syndromes, cancer patients treated with chemotherapy, prophylactic treatment to reduce the need for transfusions before major surgery) ([12]).

Considering an established safety profile of EPO in clinical practice, together with the results of experimental studies in cardiac ischemia, we designed a pilot clinical study with EPO treatment in acute MI patients. The primary objective of this single center, investigator-initiated, randomized study was to evaluate the safety and tolerability of long-acting EPO analogue darbepoetin alfa treatment in non-anemic patients with acute ST-elevation myocardial infarction (STEMI) treated with primary coronary angioplasty (PCI).

Methods

Patient population

Male patients, aged 18 to 76, with first acute STEMI and referred for PCI were considered for enrollment in the study. The inclusion and exclusion criteria are summarized in Table 1. Written informed consent was obtained from all patients before the study, and the institutional review board of the University Hospital of Groningen approved the study protocol. The study was consistent with the principles outlined in the Declaration of Helsinki. Randomization was performed by Trial Coordination Center of the Academic Hospital Groningen, based on a computer-generated randomization list. Patients were randomized to either darbepoetin alfa infusion or no infusion on a 1:1 basis. The planned number of patients to be enrolled in each group was 10.

Study design

All patients with a first STEMI and indication for PCI were screened for participation in the study. Before inclusion, medical history, physical examination and ECG were obtained. Intravenous injection of long-acting EPO analogue darbepoetin alfa (300 μg, Aranesp, Amgen Inc., Thousand Oaks, CA, USA) was administered directly before PCI. In the control arm of the study no additional medication was administered. The dose of darbepoetin was chosen on the basis of the safety and efficacy study of EPO in acute stroke ([13]). In this study, 100.000 IU of short-acting recombinant human EPO was used, with an elimination half-life of 12 hours. To achieve similar plasma EPO levels with a long-acting EPO analogue darbepoetin alfa (elimination half-life of 21 hours), we chose a 40% lower dose: 300 μg (≈ 60.000 IU). The patients were otherwise treated according to the guidelines of the European Society of Cardiology for the treatment of STEMI ([14]). After PCI, all patients were monitored on the coronary care unit of our institution for at least 24-hours, with ECG monitoring and frequent controls of blood pressure. Following the discharge from the hospital, patients underwent clinical and laboratory controls at day 10 and 30 after the MI, at the out-patient clinic of our department.

Safety parameters

Patients were closely monitored for adverse events. Besides frequent hemodynamic monitoring, each patient had complete blood cell count, blood urea nitrogen, creatinemia, electrolytes, glucose and liver enzymes evaluated at baseline (before PCI). The complete blood cell count was further closely followed and determined at time points: 24-hours, 72-hours, 10- and 30-days after PCI.

Clinical parameters

Cumulative ST-elevation was calculated as a sum of ST-elevations in 12 ECG leads, before PCI. A standard CK and CK-MB monitoring was completed in all patients. Serum EPO levels were measured at baseline, after 4, 24 and 72-hours and 10 and 30-days after PCI, using IMMULITE EPO assay (DPC, Los Angeles, California), as described before ([15,16]).

Quantification of circulating blood CD34+/CD45- cells was performed 72-hours after MI, with double labeling, with FITC-anti-CD45 and phycoerythrin-anti-CD34 monoclonal antibodies (Becton Dickinson, NJ, USA) on FACSCalibur (Becton Dickinson, NJ, USA) according to standardized procedures ([17,18]).

Four months after the admission for MI, LV function was determined by radionuclide ventriculography, using standard method. Scans were assessed by a physician blinded for treatment allocation.

Statistical analysis

Data are given as mean ± SEM, or as median ± IQR (range from the 25th to the 75th percentile) if adequate, and as frequencies for categorical variables. For the continuous variables, differences between the groups were tested using one-way analysis of variance if normally distributed, and by Mann-Whitney ranksum test if skewed distributed. For the categorical variables, the two groups were compared using a chi-square test. Correlation analysis was performed with Spearman's correlation test. We used a general linear model with repeated measures to compare the levels of serum EPO within the control group during the follow-up. All reported probability values were 2-tailed, and a p-value <0.05 was considered statistically significant. For all statistical analysis SPSS version 11.0 was used.

Table 2. Baseline characteristics

	Control group (n=10)	Darbepoetin group (n=10)	Total	P value
Age	55.7±2.9	61.7±2.9	58.7±2.1	0.17
Smoking (%)	70	20	45	0.07
History of diabetes (%)	10	10	10	1.00
Hypercholesterolemia (%)	40	20	30	0.63
History of hypertension (%)	50	40	45	1.00
Positive family history (%)	50	30	40	0.65
Anterior infarction (%)	30	50	40	0.65
Culprit lesion (%):				
LAD	30	50	40	
Cx	30	30	30	0.56
RCA	40	20	30	
Time to treatment (min)	171±14	216±21	193±14	0.10
Cumulative ST-elevation (mm)	8.0±1.1	10.6±1.5	9.4±1.0	0.19

Results

Patient characteristics

We initially included 22 consecutive, eligible patients with STEMI scheduled for PCI with stent placing. Two of them were subsequently excluded because of left main stem occlusion, requiring surgical treatment, and therefore the present population consisted of 20 (male) patients, aged 58.7±2.1 years. Other baseline characteristics are given in table 2. Study groups were comparable with respect to age, cardiovascular risk factors and culprit lesion. Slightly more patients with a history of smoking were randomized to control group (p=0.07). The longer time to treatment and higher cumulative ST-elevation suggested a more extensive ischemic damage in the group randomized to darbepoetin treatment, although the differences were not statistically significant.

Safety parameters

Both systolic and diastolic blood pressure did not increase after darbepoetin treatment and there were no differences between the groups in blood pressure nor heart rate throughout the entire 30-day long follow-up.

Selected follow-up laboratory parameters are presented in table 3. The hemoglobin and red blood cell count did not increase above the reference values in the darbepoetin-treated group. There was only a slight tendency observed towards higher levels of hemoglobin after 30 days in the patients receiving darbepoetin. As expected, the reticulocyte count increased significantly in darbepoetin-treated as compared to control group, peaking at day 3 and 10 after the MI and normalizing at day 30. Platelet count increased at day 10 in both arms of the study, with no difference between the groups. In the darbepoetin group, two patients had sustained ventricular tachycardias during 24-

Table 3. Follow-up parameters. * p<0.05 between the groups.

	Control group	Darbepoetin group
Hemoglobin (g/dl)		
Baseline	14.6±0.5	14.6±0.6
Day 1	13.9±0.5	13.9±0.6
Day 3	13.3±0.5	13.3±0.6
Day 10	13.6±0.5	14.1±0.6
Day 30	13.8±0.5	14.9±0.5
Reticulocyte count (percentile)		
Baseline	10.8±1.5	9.3±1.6
Day 1	9.9±1.4	11.1±1.4
Day 3	13.7±2.5	23.9±2.1*
Day 10	15.6±2.0	21.4±2.5
Day 30	14.1±2.0	9.2±2.2
Thrombocyte count (x10^3/µl)		
Baseline	237±18	227±16
Day 1	236±16	210±13
Day 3	248±11	212±18
Day 10	298±31	281±15
Day 30	296±30	223±22

hours post-MI and intra aortic balloon pump was temporarily used for hemodynamic stabilization. Both patients recovered and were released from the hospital after 8 and 10 days, respectively. Two patients (one in each group) underwent additional elective coronary angioplasty because of stenosis in a non-culprit vessel. Two patients (both control group) underwent coronary artery bypass graft surgery (CABG) within 4-months after MI. One patient in the darbepoetin group experienced an episode of gastrointestinal bleeding, treated endoscopically.

Clinical parameters

In the darbepoetin group, serum EPO concentration rose from baseline (9±2 U/L) to a mean of 4579±369 U/L, 4 hours after the administration, with a gradual decrease afterwards (Figure 1). Also in the control group, the serum EPO levels gradually increased from baseline (12±3 U/L) and reached a maximum 72-hours after MI (27±9, $p<0.05$). The number of CD34+/CD45- cells was significantly higher in the darbepoetin-treated group, 72-hours after MI (2.8 [1.3-6.3] vs. 1.0 [0.6-1.4] cells/µl, $p<0.01$; Figure 2).

Peak CK and CK-MB were both higher in the darbepoetin-treated group, although this difference did not reach statistical significance (median values; peak CK: 1077 [490-1910] vs. 487 [188-1339] U/l, p=0.22; and peak CK-MB: 101 [49-155] vs. 47 [17-92] U/l, p=0.09).

After 4 months, LV ejection fraction was not different between darbepoetin and control group (52±3 vs. 48±5%, p=NS). However, because two patients in the control arm treatment underwent CABG surgery during this period, they were not included in the analysis.

We also compared enzymatic infarct size to LV function at 4-months, and studied correlations.

Figure 1. Serum erythropoietin levels during 30-day follow-up. *p<0.05 between groups. †p<0.05 for repeated measures analyses within the control group.

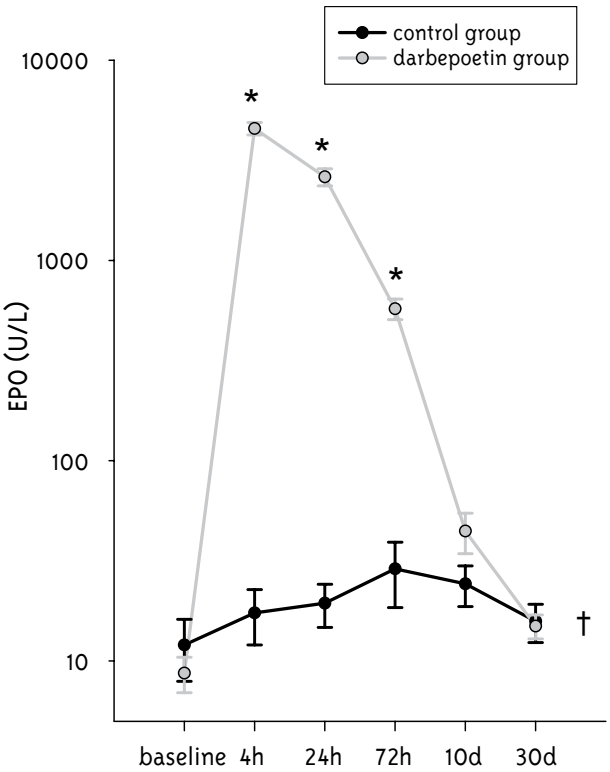


Figure 2. Number of CD34+/CD45- cells, 72-hours after MI. Box plots show the median with 25-75% range.

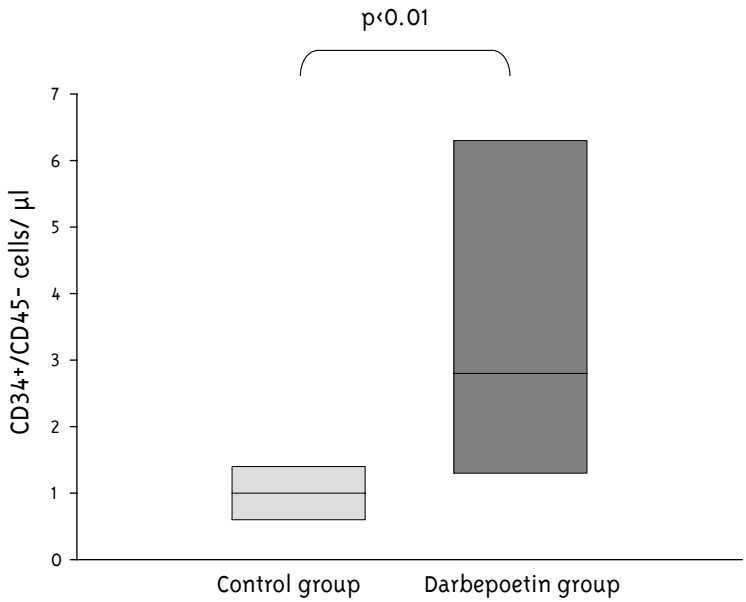
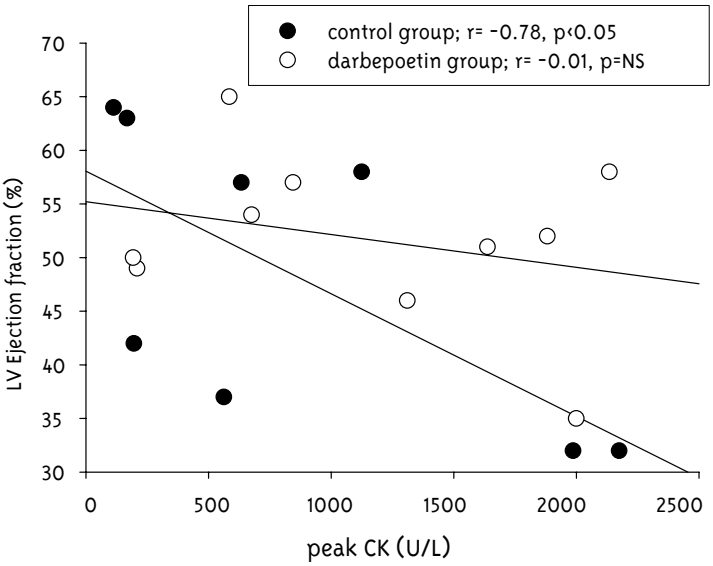


Figure 3. Correlations between peak CK levels after MI and LV-ejection fraction measured 4-months later.



In the control group, a significant inverse correlation between peak CK value and LV ejection fraction was observed (r=-0.78, p<0.05). In the darbepoetin group, there was no such correlation present (r=-0.01, p=NS; Figure 3).

Discussion

The main finding of the present pilot study is that a single bolus of long-acting EPO analogue darbepoetin alfa in patients with first acute STEMI appears to be safe and well tolerated. No hypertensive, thromboembolic or other serious adverse events were observed during the 30-day long follow-up. Darbepoetin treatment led to a significant elevation of CD34+/ CD45- cells, 72-hours after the drug administration. Despite non-significantly longer "time to treatment" and more extensive baseline area at risk (cumulative ST-elevation) in the darbepoetin-treated group, LV function 4 months after the MI was similar in both groups.

The present study is the first to examine the effect of EPO in acute MI. Indeed, while there is a rapidly increasing number of studies which suggest a protective role of EPO treatment in experimental cardiac ischemia, so far no clinical data are available. Recently, high levels of *endogenous* EPO in patients with first MI who underwent successful PCI, were found to be associated with smaller infarcts, which was interpreted by the authors as a possible endogenous, protective mechanism ([19]). In the control group of the present study, the *endogenous* serum EPO levels also gradually increased after the MI, reaching a maximum over 72-hours.

Besides protecting against acute ischemic injury, EPO has been shown to induce

neovascularization over a longer time period ([20]). We have evaluated this issue in a rat model of post-MI heart failure ([21]). EPO treatment improved cardiac function, which was associated with increased capillary density and capillary-to-myocyte ratio, indicating formation of new blood vessels.

The mechanism behind the effect of EPO on neovascularization remains largely unknown. Both stimulation of *in situ* endothelial cells proliferation or mobilization of EPCs derived from the bone marrow may play a role. Recently, increased levels of circulating EPCs were associated with reduced risk of death from cardiovascular causes in patients with confirmed coronary artery disease ([22]). EPO was shown to stimulate the mobilization and functional activity of EPCs ([23]). In the BOOST trial, intracoronary infusion of autologous CD34+ bone marrow cells after myocardial infarction improved the global LV ejection fraction 6 months after cell transfer ([24]). Administration of darbepoetin in the present pilot study led to almost a 3-fold increase in the number of circulating CD34+/CD45- cells, measured 72-hours after MI.

We observed non-significantly higher peak CK and CK-MB levels in patients randomized for darbepoetin treatment. The most probable explanation for this finding is the longer "time to treatment" in the darbepoetin-treated group, as shown in the study by Liem *et al.* ([25]), in which comparable time delay (ca. 40 minutes) led to a significantly larger enzymatic infarct size and lower LV ejection fraction at 6 months. However, despite higher peak CK in patients treated with darbepoetin, the LV ejection fraction 4 months after the MI was similar in both groups. In numerous studies, enzymatic infarct size significantly correlates with LV function assessed at various time points after the MI([26-28]). Because a linear relationship exists between peak CK levels after acute MI and LV ejection fraction during the follow-up ([26]), we calculated the correlations in both groups of patients. While we observed a strong inverse correlation between peak CK value and LV ejection fraction in the control group, with comparable linear regression equations as previously described ([28]), no such correlation was detected in darbepoetin group. This may suggest a positive effect of darbepoetin treatment on longer-term infarct healing and/or cardiac remodeling. While the present study was conducted in patients with normal hemoglobin levels, recent studies showed that cardiovascular mortality in patients with acute coronary syndromes increases as hemoglobin levels become lower ([29,30]). Specific therapeutic strategies, including EPO treatment, in such anemic patients with MI should also be further considered.

To date the only clinical study evaluating the non-hematopoietic effects of EPO was performed in stroke patients ([13]). In this first clinical, randomized, double-blind trial, EPO was given to patients with ischemic stroke presenting within 8 hours after the onset of symptoms. In spite of the relatively small number of patients in this study (n=40), EPO administration in high-doses (entire dose 100.000 IU/ given in three days) proved to be both safe and beneficial. Patients randomized to the EPO group showed significant improvement in clinical outcome parameters and a trend toward smaller infarct sizes, assessed by MRI. Furthermore, the serum levels of brain infarct damage marker (S100β) were significantly attenuated in EPO-treated group. Currently, a larger, multicenter "EPO stroke study" is being carried out in Germany ([31]).

The dose of darbepoetin used in our study was based on the results of experimental studies, as well as on the basis of the above-mentioned EPO study in acute stroke ([13]). However, because of a longer elimination half-life of darbepoetin alfa we chose a single high-dose darbepoetin infusion instead of multiple EPO administrations used in stroke patients. Importantly,

the measured serum EPO levels (4000-6000 U/L) were similar in both studies.
In cardiology, EPO has been used to correct anemia in patients with chronic heart failure (CHF). In a number of small studies, normalization of hemoglobin levels in these patients was associated with improved LV ejection fraction ([32]) and enhanced exercise capacity ([33]). While this amelioration could be partly attributed to hemoglobin elevation and thus increased oxygen-binding capacity of blood, the non-hematopoietic effects of EPO must also be considered.
Although treatment with EPO is generally well tolerated and safe, it may be associated with adverse effects ([34]). Rapid increases in hematocrit may cause hypertension ([12]), which develops in 20-30% of renal patients treated with EPO ([35]). Postulated mechanisms for EPO-induced hypertension include increased viscosity or vasoconstrictive responses due to the correction of anemia ([34]). Furthermore, EPO could also have direct vasopressor effect at the level of small resistance vessels ([36]). In our study, both systolic and diastolic blood pressure was lower during the whole follow-up in the group of patients treated with darbepoetin, as compared to controls. An other potential side effect is the increase in the number of thrombotic events ([37]), and EPO was also shown to increase the platelet count by 10-20% ([38]). However, most, if not all, of the above-mentioned complications are associated with an increase in hematocrit levels, which occurs mainly during chronic EPO therapy. With respect to the present study, administration of a single dose darbepoetin will generally not lead to a significant increase in hematocrit, but merely support the restoration of hematocrit levels after MI. In addition, we did not observe any thrombotic complications or elevation of platelet count in the group treated with darbepoetin. Thus, by avoiding adverse effects of chronic therapy, EPO administration remains attractive in a setting of acute MI.
In conclusion, high-dose darbepoetin treatment in acute MI is both safe and well tolerated. Darbepoetin treatment after MI stimulates EPCs mobilization. A larger scale clinical trial is warranted to assess the possible beneficial effect of EPO treatment in patients presenting with acute cardiac ischemia.

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