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Erythropoietin in cardiac ischemia

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Chapter 4

Erythropoietin induces neovascularization and improves cardiac function in rats with heart failure after myocardial infarction

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Abstract

Objectives: We assessed the effects of erythropoietin (EPO) treatment in a rat model of post-myocardial infarction (MI) heart failure.
Background: EPO, traditionally known as a hematopoietic hormone, has been linked to neovascularization. Whereas administration of EPO acutely after MI reduces infarct size and improves cardiac function, its role in the failing heart is unknown.
Methods: Rats underwent coronary ligation or sham surgery. Rats with MI were randomly assigned to: untreated (MI), a single bolus of EPO immediately after MI induction (MI-EPO-early), EPO treatment immediately after MI and once every three weeks (MI-EPO-early+late) and EPO treatment starting three weeks after induction of MI, once every three weeks (MI-EPO-late). After nine weeks, hemodynamics, infarct size, myosin heavy chain (MHC) isoforms, myocyte hypertrophy and capillary density were measured.
Results: EPO treatment started immediately after MI (MI-EPO-early and MI-EPO-early+late) resulted in a 23-30% reduction in infarct size ($p<0.01$), and accordingly hemodynamic improvement. EPO treatment, started three weeks after MI (MI-EPO-late), did not affect infarct size, but resulted in an improved cardiac performance, reflected by a 34% reduction in left ventricular end-diastolic pressure ($p<0.01$), and 46% decrease in atrial natriuretic peptide levels ($p<0.05$). The improved cardiac function was accompanied by an increased capillary density ($p<0.01$), an increased capillary-to-myocyte ratio ($p<0.05$) and a partial reversal of β-MHC ($p<0.05$) in all treated groups.
Conclusion: In addition to its effect on infarct size reduction, EPO treatment improves cardiac function in a rat model of post-MI heart failure. This observation may be explained by neovascularization, associated with an increased α-MHC expression.

Introduction

Erythropoietin (EPO) is best known as a hematopoietic growth factor, promoting proliferation and differentiation of erythroid progenitor cells. However, the expression of the EPO-receptor outside the hematopoietic system, including endothelial cells, cardiomyocytes and neurons, may suggest additional effects of EPO beyond hematopoiesis ([1-4]).
Because an insufficient amount of capillaries may lead to left ventricular (LV) dilation and heart failure after myocardial infarction (MI) ([5]), treatment directed towards increasing capillary density might be beneficial in heart failure. Expanding evidence shows that EPO is involved in angiogenesis. It has been shown that stimulation of cultured endothelial cells with EPO resulted in cell proliferation, chemotaxis and differentiation into vascular structures ([6]). Furthermore, Jaquet *et al* found that EPO and vascular endothelial growth factor (VEGF) were equally effective in stimulating angiogenesis in endothelial cells derived from the myocardium ([7]). Most recently, it has been shown that EPO treatment in a rat stroke model resulted in an increased capillary density around the ischemic lesion ([8]).
In addition, EPO has been implicated to play a protective role during acute ischemia in brain ([2;9;10]) and heart ([11-13]). Pretreatment with exogenous EPO rescued hypoxic cultured cardiomyocytes from apoptosis ([12]). EPO perfusion during ex-vivo ischemia-reperfusion improved left ventricular function and reduced cellular damage([4;13;14]). Acute, systemic treatment with EPO, in a rodent ischemia-reperfusion model, substantially reduced infarct size and decreased

myocardial apoptosis ([12]), even when EPO was administered after reperfusion([11;15]).
While the cardioprotective effects of EPO during acute MI are increasingly recognized, the role of EPO treatment in chronic heart failure (CHF) is unknown. Therefore, we assessed the effects of EPO treatment in a rat model of post-MI heart failure ([16]). In this model, induction of MI leads to a time and infarct size related ventricular dilatation and heart failure ([17]). We hypothesized that EPO treatment initiated after heart failure development (three weeks after induction of MI) would improve cardiac performance, possibly by increasing capillary density. To distinguish the acute effects of EPO (i.e. infarct size reduction) from its effects in CHF, we studied two additional groups. In one group we administered only a single dose of EPO immediately after MI, and in a second group we administered EPO immediately after MI and continued EPO treatment during the experiment.

Methods

Animals

We used male Sprague Dawley rats weighing 270-330 g (Harlan, Zeist, The Netherlands). Animals were fed ad libitum, and housed in groups of four to five rats, according to institutional rules with 12:12 hours light-dark cycles. The experimental protocol was approved by the Animal Ethical Committee of the University of Groningen.

Design of the study

Rats were either subjected to left coronary artery ligation (n=85) or sham surgery (n=8). Rats with MI were randomized to one of four groups; untreated (MI) or three different treatment strategies with EPO: a single bolus of EPO immediately after ligation (MI-EPO-early), EPO treatment directly after ligation and once every three weeks (MI-EPO-early+late) and EPO treatment starting three weeks after ligation, once every three weeks (MI-EPO-late). EPO (Darbepoetin-alpha; Aranesp, Amgen Inc., Thousands Oaks, CA, USA) was administered intraperitoneally at a dose of 40 μg/kg, which equals 8.000 U/kg recombinant human EPO (Amgen Inc.) and is in close range of known dosages for organ protection ([11;12;18]). Hematocrit was measured at baseline and at week one, three, four, six and nine after surgery. Persons blinded to the treatment groups performed the analysis of samples obtained from the experiments.

Myocardial infarction model

This model has been described previously ([16]). Briefly, rats were anesthetized with 2% isoflurane in 1.0 L oxygen/minute. After intubation, the rats were put on a mechanical ventilator (frequency 90/min) and a left-side thoracotomy was performed. MI was induced by ligating the proximal portion of the left coronary artery, beneath the left atrial appendage. In sham operated rats, the same surgery was performed, without ligating the suture.

Hemodynamic measurements

After nine weeks rats were anaesthetized as described above. Microtip pressure transducer (Millar Instr. Inc., Houston, Texas) was inserted into the left ventricular cavity via the right carotid artery. After a 3-min period of stabilization, heart rate (HR), left ventricular systolic

Table 1. Characteristics of the experimental groups

	Sham	**MI**	**MI-EPO-early**	**MI-EPO-late**	**MI-EPO-early+late**
General					
n	8	12	12	13	13
Infarct size (% of LV)	...	43±3	$30\pm2^{\S}$	41±3	$33\pm2^{\S}$
Hemodynamics					
Heart rate (bpm)	313±8	324±6	332±7	326±7	328±8
SBP (mmHg)	127±3	$111\pm4^{\dagger}$	$115\pm3^{\dagger}$	$120\pm3^{\ddagger}$	$122\pm3^{\ddagger}$
DBP (mmHg)	78±2	78±2	79±2	83±3	86±2
Body/organ weight					
BW (g)	390±10	395±11	401±8	400±7	421±6
Lungweight/BW (mg/g)	3.9±0.1	$6.4\pm1.0^{\dagger}$	$4.2\pm0.5^{\S}$	$3.9\pm0.1^{\S}$	$3.9\pm0.2^{\S}$
Heartweight/BW (mg/g)	3.2±0.1	$4.0\pm0.2^{\dagger}$	$3.8\pm0.1^{\dagger}$	$3.7\pm0.1^{*}$	$3.7\pm0.1^{*}$
Hematocrit					
baseline (%)	48±0.6	47±0.3	48±0.5	48±0.6	47±0.6
1 week (%)	48±0.6	47±1.1	$58\pm0.9^{\dagger,\S}$	46±0.7	$59\pm0.7^{\dagger,\S}$
3 weeks (%)	50±1.1	49±0.7	$53\pm0.5^{\dagger,\S}$	49±0.7	$53\pm0.7^{\dagger,\S}$
4 weeks (%)	50±0.5	51±0.8	50±0.4	$62\pm0.5^{\dagger,\S}$	$64\pm1.8^{\dagger,\S}$
6 weeks (%)	50±0.4	50±0.7	49±0.6	$60\pm0.7^{\dagger,\S}$	$61\pm0.7^{\dagger,\S}$
9 weeks (%)	44±0.5	44±1.5	44±0.7	$54\pm1.3^{\dagger,\S}$	$56\pm1.7^{\dagger,\S}$

Data are presented as mean ± SEM. n indicates number of animals; LV, left ventricle; SBP, systolic blood pressure; DBP, diastolic blood pressure; BW, bodyweight. $^{*}p<0.05$; $^{\dagger}p<0.01$ vs. Sham; $^{\ddagger}p<0.05$, $^{\S}p<0.01$ vs. MI.

pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), and developed left ventricular pressure (dLVP=LVSP-LVEDP) were measured. As indices of contractility and relaxation, the maximal rates of increase and decrease in LVP ($+dP/dt_{max}$ and $-dP/dt_{max}$) were determined. The catheter was retracted into the aortic arch and arterial systolic and diastolic blood pressures (SBP, DBP) were recorded.

Plasma N-terminal ANP levels

Arterial blood was collected after nine weeks, anti-coagulated with EDTA, and plasma was stored at −80°C until assayed. Plasma N-terminal atrial natriuretic peptide (N-ANP) was measured by a commercially available radioimmunoassay (Biotop, Oulu, Finland), as described previously ([19]).

Infarct size and left ventricular hypertrophy

After hemodynamic measurements, hearts were rapidly excised and weighed. Mid-papillary slices were prepared for immunohistochemistry. Slices were fixed in 4% paraformaldehyde and paraffin-embedded. Infarct size was determined by planimetry at mid-ventricular levels in transverse slices on picrosirius red/fast green–stained sections. Infarct size was expressed as the percentage of scar length to total left ventricular circumference, as described previously([20;21]). Deparaffinized 5-µm thick sections were stained with a Gomori's silver staining, in order to visualize individual myocytes in the viable LV wall, the area with the most pronounced underperfusion ([22]). Using image analysis (Zeiss KS 400, Jena, Germany), concentric myocyte hypertrophy in the viable LV wall, remote from the infarcted area, was measured as the cross-sectional area of transversally cut myocytes showing a nucleus. Myocyte density was calculated as the average number of myocytes per tissue area. In each stained section, measurements were averaged from three different counting fields (± 75 myocytes per heart).

Myosin Heavy Chain (MHC) Isoform Analysis

Samples of the left ventricle (not infarct area), were frozen in liquid nitrogen and stored at –80°C. The freeze dried samples were dissolved in a buffer and gel electrophoresis was performed as described previously ([23]). Samples (0.5μg) were run at constant current (24 mA) for 5 hours. Silver staining of the gels and laser scanning densitometry was performed to identify differences in myosin isoform composition (i.e. α-MHC and β-MHC).

Capillary density

To visualize capillaries in the myocardium in the same area as used for the measurements of the myocyte size, endothelial cells were stained with biotin-labeled Lectin GSL (1:100; Sigma-Aldrich, St. Louis, Missouri), as previously described ([16]). Since Lectins stain not only capillaries but also other vessels, a size criterion of 10 μm was used to exclude small arterioles and venules. Image analysis (Image Pro-plus version 4.5, Media Cybernetics Inc.,Silver Spring, Maryland) was used to measure capillary density, calculated as the number of capillaries per tissue area. The measured total tissue area was corrected for the remaining interstitial space. Actual neovascularization was derived from an increased capillary-to-myocyte ratio, which has been calculated as capillary density divided by myocyte density ([24]).

Statistical analysis

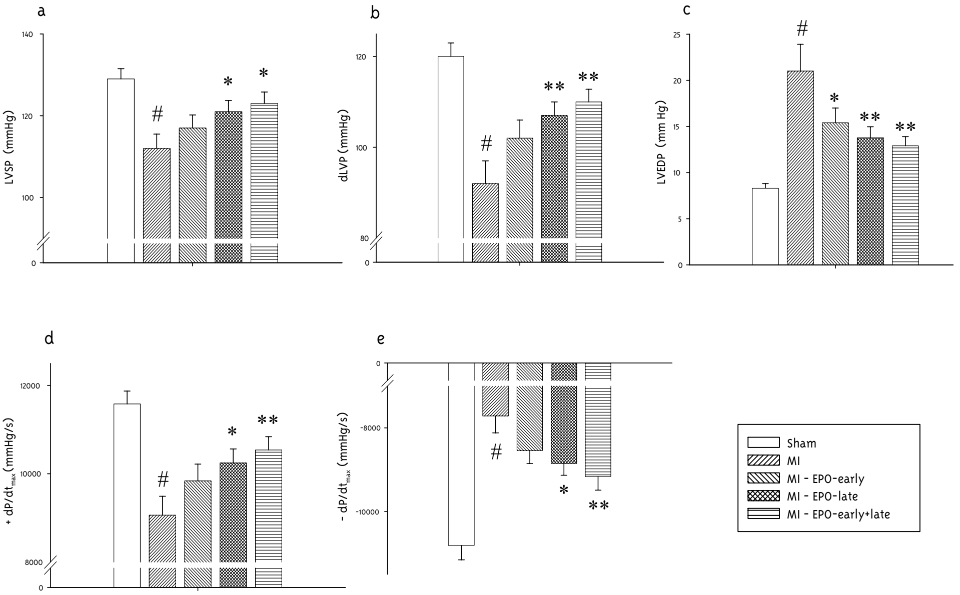
Data are presented as mean ± SEM. Statistical analysis between groups was performed by 1-way ANOVA. When a statistically significant difference was detected, a Fisher's protected LSD post-hoc analysis was performed. Correlation analysis was performed with Pearson's correlation tests. Differences were considered significant at $p<0.05$.

Results

General

Overall mortality after MI was 41%. Mortality occurred only in the first 24 hours after induction of MI. There were no statistically significant differences in mortality between the four groups (MI:50%, MI-EPO-early: 40%, MI-EPO-late: 32% and MI-EPO-early+late: 41%; p=0.54). No mortality was observed in sham-operated rats. At baseline, no differences in body weight were observed (data not shown). General characteristics after nine weeks are shown in table 1. Body weight was comparable among the five groups. Among groups with MI, systolic blood pressure (SBP) was significantly lower only in MI and MI-EPO-early compared to sham; $p<0.01$. SBP was significantly higher in MI-EPO-late and MI-EPO-early+late, compared to MI group ($p<0.05$). No significant differences were observed in heart rate and diastolic blood pressure (DBP), although there was a trend towards higher DBP in the groups

Figure 1. Effects of myocardial infarction and EPO treatment on hemodynamic parameters. LVSP indicates left ventricular systolic pressure; dLVP, developed left ventricular pressure; LVEDP, left ventricular end diastolic pressure; $+dP/dt_{max}$ and $-dP/dt_{max}$, maximal rate of increase and decrease of ventricular pressure, respectively. * $p<0.05$ vs. MI, ** $p<0.01$ vs. MI, # $p<0.01$ vs. Sham



repeatedly treated with EPO (MI-EPO-late and MI-EPO-early+late). The changes of the hematocrit throughout the experiment are also shown in table 1. After nine weeks hematocrit values were significantly elevated in the MI-EPO-late and MI-EPO-early+late, compared to other groups.

Infarct size

LV-infarct size (% of LV) was comparable between MI and MI-EPO-late, 43% and 41% respectively ($p=0.60$; table 1). Treatment with EPO immediately after coronary artery ligation, reduced infarct size by 30% in MI-EPO-early and by 23% in MI-EPO-early+late group (both $p<0.01$ vs. MI; table 1).

Hemodynamic measurements

Hemodynamic data obtained nine weeks after surgery are summarized in figure 1. LVSP and developed LVP (dLVP) were both clearly diminished in MI compared to sham operated rats ($p<0.01$ for both). MI-EPO-late and MI-EPO-early+late showed a significantly higher LVSP and dLVP, compared to MI (both $p<0.05$). One single bolus of EPO immediately after ligation (MI-EPO-early) did not result in a significantly improved LVSP or dLVP (figure 1A and 1B).
LVEDP was elevated in MI compared to sham operated rats (21±3mmHg vs. 8±1mmHg; $p<0.01$). Importantly, EPO treatment started three weeks after MI (MI-EPO-late), resulted in a 34% decrease in LVEDP, compared to MI ($p<0.01$), despite similar infarct sizes. Immediate treatment with EPO after induction of MI (MI-EPO-early and MI-EPO-early+late) led to a 27% and 38% reduction in LVEDP respectively, compared to MI group ($p<0.05$ and $p<0.01$;

Figure 2. Plasma N-terminal ANP levels. * p<0.05 vs. MI, ** p<0.01 vs. MI, # p<0.01 vs. Sham.

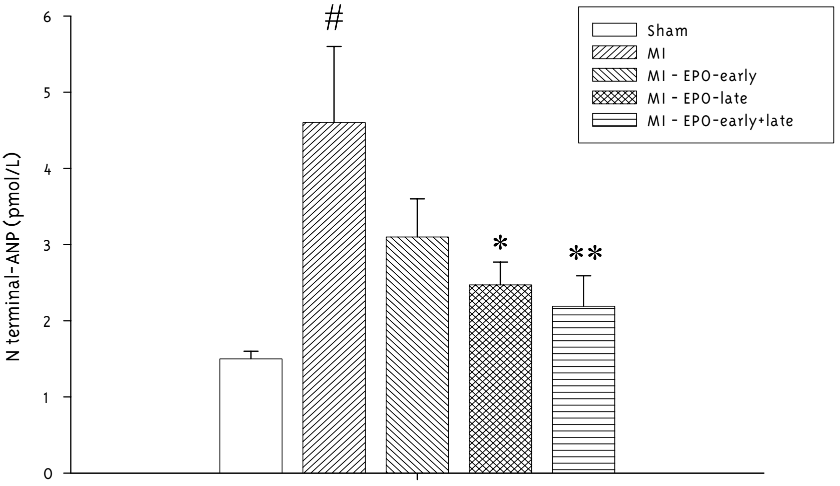


figure 1C).

Myocardial contractility (+dP/dt$_{max}$) and myocardial relaxation (-dP/dt$_{max}$) were both impaired in MI compared to the sham group (both $p<0.01$). MI-EPO-late and MI-EPO-early+late showed an improved contractility and relaxation compared to MI (all $p<0.05$). In contrast, when only one single bolus of EPO was administered immediately after MI (MI-EPO-early), contractility and relaxation were not significantly improved compared to MI (figure 1D and 1E).

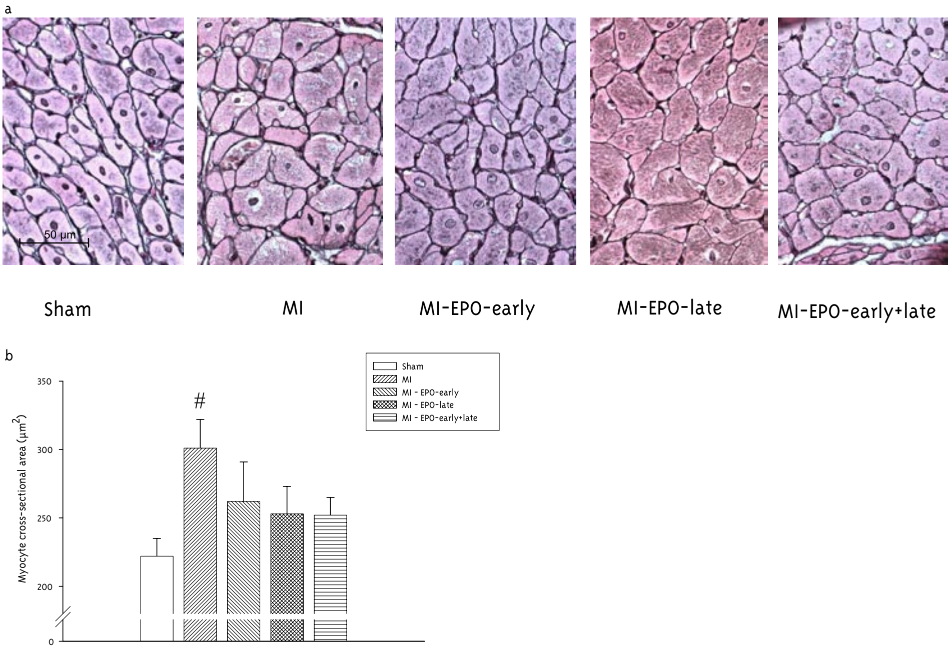
N-terminal ANP levels

Figure 2 shows that plasma N-ANP levels were three-fold increased in MI group ($p<0.01$ vs. sham-operated animals). Furthermore, N-ANP levels were significantly reduced in the MI-EPO-late and MI-EPO-early+late groups (both $p<0.05$ vs. MI), returning to sham values (both p=NS vs. sham). The MI-EPO-early group showed a trend towards lower N-ANP levels ($p=0.07$ vs. MI).

Organ weights and LV hypertrophy

As shown in table 1, the ratios of heart weight (HW) to body weight and that of lung weight to body weight were significantly increased in the MI compared to the sham-operated group (both $p<0.01$). Lung weight to body weight (an indirect expression of the LV-end diastolic pressure and thus severity of heart failure) was significantly reduced in all EPO treatment groups (all $p<0.01$ vs. MI). A trend towards lower HW to body weight compared to MI was observed in MI-EPO-late and MI-EPO-early+late groups. LV hypertrophy was further studied by histological analysis. Representative photomicrographs of Gomori stained sections of the viable LV free wall are shown in figure 3A. MI resulted in a 35% increase in myocyte cross-sectional area, compared to sham ($p<0.05$). All EPO treated groups showed a trend towards a smaller myocyte cross-sectional area, although this did not reach statistical significance (figure 3B).

Figure 3a) Gomori stained sections in the LV viable wall of the five different groups, showing individual myocytes. b) Bar graphs showing the actual measurements for the myocyte cross-sectional area in the different experimental groups. # p<0.05 vs. Sham.



Differences in MHC isoform composition

Relative proportion of cardiac α-MHC and β-MHC were compared in LV protein samples between the five different groups. MI resulted in a more than 5-fold increase in expression of β-MHC, compared to sham operated rats ($p<0.01$). EPO treatment in all three groups reduced the expression of β-MHC by 26-31%, compared to MI ($p<0.05$; figure 4).

Capillary density

Capillaries stained with lectin were clearly discernable in the myocardium. Figure 5A shows representative photomicrographs of the five different groups. Capillary density was significantly reduced in the MI group compared to the sham-group ($p<0.01$). EPO treatment in all three groups prevented the decrease in capillary density after induction of MI and restored it to sham values, as shown in Figure 5B (p=NS vs. sham). Furthermore, in the MI-EPO-late and MI-EPO-early+late groups, we observed a 39% and 48% increase in capillary-to-myocyte ratio, respectively (both $p<0.05$ vs. MI), whereas MI-EPO-early showed a clear trend ($p=0.05$ vs. MI) towards an increased capillary-to-myocyte ratio (figure 5C).

In order to relate LV functional parameters through a MHC-shift to increased capillarization, correlations were determined. We observed a strong correlation between capillary density and β-MHC expression ($r=-0.47$, $p<0.01$) and subsequently between β-MHC expression and cardiac contractility and relaxation, $r=-0.52$ and $r=0.61$ respectively (both $p<0.01$). Furthermore, capillary density was correlated with myocardial contractility ($r=0.32$) and relaxation ($r=-0.37$; both $p<0.05$).

Figure 4. Effects of myocardial infarction and EPO treatment on β-MHC protein expression as a percentage of total MHC expression. * $p<0.05$ vs. MI, # $p<0.01$ vs. Sham.

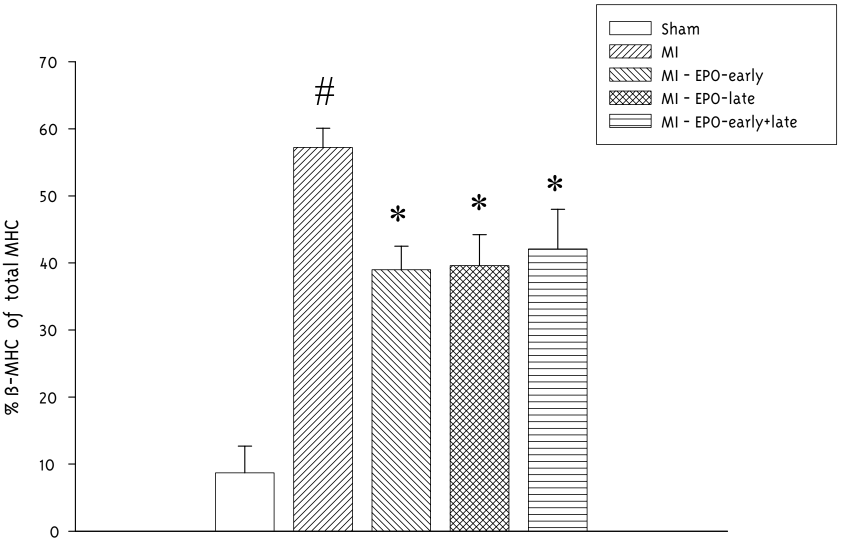
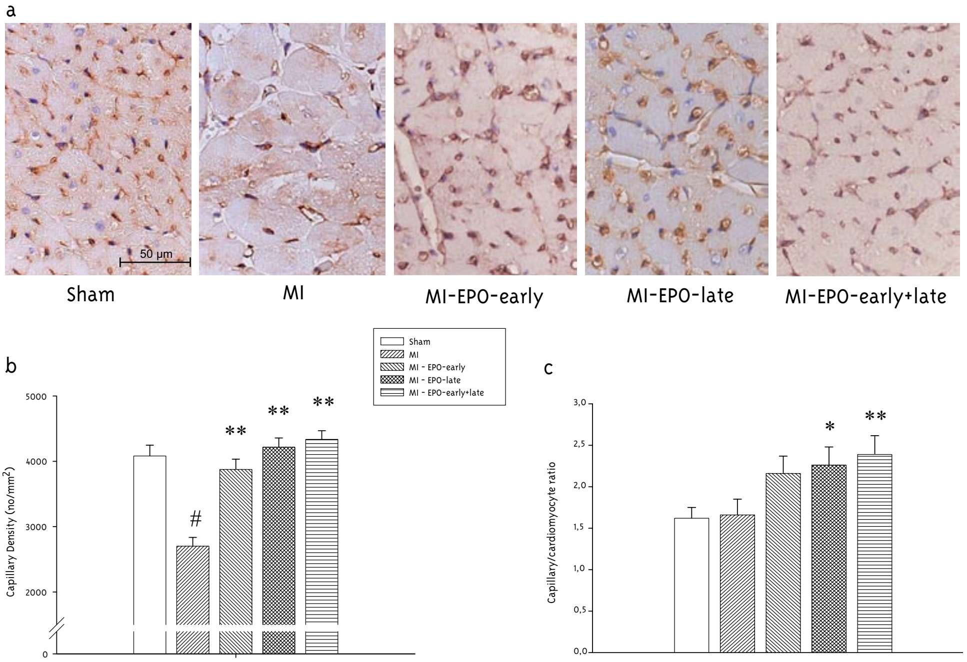


Figure 5a) Tissue sections stained with lectin in the viable free wall of the five different groups, showing individual capillaries. b) Actual measurements for capillary density in number of capillaries per mm^2. c) Bar graphs representing the capillary-to-myocyte ratio in the different treatment groups. * $p<0.05$ vs. MI, ** $p<0.01$ vs. MI, # $p<0.01$ vs. Sham.



Discussion

In the present study, the effects of EPO treatment in a rat model of post-MI heart failure were examined. To our knowledge, this study shows for the first time that EPO treatment initiated three weeks after induction of MI results in an improved cardiac function, as shown by a 17% increase of dLVP at 34% reduction in LVEDP and a 46% decrease in N-ANP levels. Furthermore, our data indicate that EPO restores capillary density to sham levels and increases the capillary-to-myocyte ratio, indicating neovascularization.

Myocardial structure and cardiac function

Previous studies already revealed that EPO has ancillary properties besides hematopoiesis. One of the first studies on EPO in the heart showed that EPO injected intraperitoneally for seven days, reduced cardiomyocyte loss by 50% after ischemia-reperfusion injury ([25]). These observations have been confirmed by others. Parsa *et al*. showed a 25% reduction in infarct size after 4 days of permanent occlusion of the left circumflex coronary artery in rabbits([12]). A single dose of EPO at the onset of MI reduced infarct size, which was accompanied by reductions in LV size and an improved LV ejection fraction, measured by echocardiography, during eight weeks follow-up ([26]). Our results are in line with these findings; one single dose of EPO administered immediately after induction of MI, reduces infarct size by 30% and improves hemodynamics. Mechanisms behind this acute protective effect of EPO may be related to its anti-apoptotic effect. We and others showed that in the acute phase of MI, EPO markedly prevents cardiac cells from undergoing programmed cell death (apoptosis)([11;12;15;26]). After MI, apoptosis is first observed in endothelial cells of small coronary vessels, spreading to the surrounding cardiomyocytes ([27]). Since the EPO-receptor is predominantly expressed on endothelial cells, preventing apoptosis in these cells may rescue the underlying myocardium ([13]). Recently, it has been postulated that cardiac fibroblasts may also play a role in the cardioprotective effects of EPO ([15]).

Although a single dose of EPO clearly improves cardiac performance, prolonged EPO treatment (MI-EPO-early+late) was associated with a further restoration of cardiac function. Mechanisms involved in this process are most likely distinct from its acute cardioprotective effect. This is clearly demonstrated by the finding that EPO treatment, initiated three weeks after MI, although not reducing infarct size, significantly improves cardiac function, reflected by a 17% increase of dLVP at 34% decrease in LVEDP, and restoring N-ANP levels to sham values. Since the effect of EPO treatment in this group could not be explained by infarct size reduction, other properties of EPO should be considered to elucidate the observed beneficial effects of EPO in heart failure.

Neovascularization

EPO has been shown to possess proangiogenic properties. As discussed above, the EPO-receptor is expressed on endothelial cells and EPO has been shown to stimulate the proliferation and migration of endothelial cells *in vitro* ([6]). Additional experiments in chick embryos demonstrated that EPO treatment results in angiogenesis similar to other well-known angiogenic cytokines ([6]). Furthermore, EPO induces vascular sprouting in a rat aortic ring model ([28]). In human cultured myocardial tissue, EPO stimulates capillary outgrowth comparable to VEGF ([7]). In a rodent model of hind-limb ischemia, EPO increases capillary density 1.6-fold ([29]). In a rat model of chronic renal failure, characterized by left ventricular hypertrophy and capillary deficiency, EPO treatment results only in a small non-significant increase in cardiac capillary density (30). In a rat model of stroke, EPO treatment initiated 24 hours after infarction, enhances angiogenesis and improves neurological function, while it does not significantly influence infarct size. Our results suggest a similar effect of EPO in the heart. We find that EPO treatment restores capillary density to sham values and increases capillary-to-myocyte ratio, indicating actual capillary growth (24), which is more pronounced in the groups with prolonged EPO treatment.

To study the functional consequences of increased capillarization, we examined the expression of different MHC isoforms in heart tissue. Cardiomyocytes express both fast α-MHC and slow β-MHC isoforms, which differ on the basis of ATPase activity. Recently, it has been shown that expression of a small amount of α-MHC (~12%) in rat cardiomyocytes significantly increases power output, indicating that a small shift in MHC composition as we found in all EPO treated groups may improve contractility(31). Increased capillary density was significantly correlated with the percentage of β-MHC isoform as well as with myocardial function ($+dP/dt_{max}$ and $-dP/dt_{max}$), providing a link between neovascularization and functional effects of EPO.

The mechanism behind the effect of EPO on new blood vessel formation in the heart remains unknown. In general, stimulation of in situ endothelial cell proliferation or bone marrow-derived endothelial progenitor cells (EPCs) might play a role. Previous work showed that EPO effectively increases the amount of circulating EPCs (32), and significantly induces angiogenesis (29). Future experiments are needed to delineate the mechanism of EPO stimulated capillary growth.

Hematopoietic effect

Another important property of EPO that might be involved in the cardioprotective effect observed in our study, is its hematopoietic effect. Human recombinant erythropoietin increases the number of reticulocytes after administration to rats after 3-4 days with maximum after 8-11 days (33). In our study, we observed significant hematocrit elevation one week after a single dose of EPO. In the groups treated with multiple EPO doses, hematocrit remained significantly elevated throughout the experiment. The beneficial effects seen in these groups might thus, in part, be explained on the basis of increased oxygen-carrying capacity of blood. However, the effects of higher red blood cell mass on oxygen delivery is not straightforward, since elevated hematocrit may downregulate nitric oxide synthesis and thus impair tissue blood flow (34). In the clinical setting, increasing the number of red blood cell mass by blood transfusion has been reported to improve outcome in elderly patients after acute MI (35). Nevertheless, this beneficial effect is only seen in patients with hematocrit <33%. On the other hand, reduction in the infarct size observed in the early treated groups, could not be attributed to the hematopoietic effect of EPO, since cell death and MI expansion occur mainly during the first 3 days after ischemic insult (26) and thus before significant hematocrit elevation.

Conversely, an increase in hematocrit may itself tend to deteriorate myocardial perfusion through adverse rheological effects. Elevated hematocrit levels (up to 80%) in polyglobulic mice, overexpressing EPO, enlarge cerebral infarct volumes and leukocyte infiltration after permanent occlusion of middle cerebral artery (36). Furthermore, EPO administration and consequent higher hematocrit has been associated with other adverse cardiovascular effects. Therapeutic levels of EPO may cause higher incidence of thrombosis (37) and could lead to blood pressure elevation (38). In the present study, rats repeatedly treated with EPO, had a higher systolic blood pressure. This increase might be related to the improved cardiac function; however, the systolic blood pressure remained below the values observed in the sham operated group.

Clinical Implications

In clinical settings, EPO treatment has already been used to correct anemia in patients with CHF. Anemia is frequently observed in patients with CHF and related to increased morbidity and mortality ([39;40]). Furthermore, not only anemia, but also elevated endogenous EPO levels are independently associated with an impaired outcome in CHF ([41]). Normalization of hemoglobin levels in mild anemic patients with CHF has a positive effect on LV ejection fraction ([42]) and peak VO_2 ([43]). In addition to correction of anemia, other non-hematopoietic effects of EPO may play a role in the improvement observed in patients with CHF treated with EPO.

Besides the treatment of anemia, EPO is currently under investigation for its neuroprotective properties. In the first clinical, randomized, proof-of concept trial, EPO was given to patients with ischemic stroke ([44]). 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 towards smaller infarct sizes.

However, chronic therapy with EPO is also associated with adverse effects related to hematocrit elevation, such as hypertension and tromboembolic complications. This could be overcome by using a lower dose of EPO, as shown by Bahlmann *et al.* ([45]). In this study, low-dose of darbepoetin (0.1 μg/kg/week) rendered tissue protection in the kidneys even without raising hematocrit levels. The recently discovered non-hematopoietic derivates of EPO, retaining tissue protection, but without the undesired effects on hematopoiesis may become another possibility for chronic administration ([46]).

Limitations

Several limitations of the present study have to be acknowledged. Although a clear increase in capillary density and capillary-to-myocyte ratio was observed, the improvement of cardiac function might also be related to other effects of EPO treatment. Since we did not perform sequential measurements of cardiac function, further studies would be needed to specifically denote the time-dependent effect of EPO treatment on attenuation of heart failure development.

We did not measure the direct myocardial perfusion, and therefore functional evidence of an improved perfusion remains unclear. However, we observed a clear correlation between capillary density and β-MHC expression and cardiac function. Furthermore, we used the Fisher's LSD post-hoc statistical test for analyzing our data, which does not control for multiple comparisons.

Conclusion

In summary, the present study demonstrates that EPO treatment in a rat model of heart failure improves cardiac function beyond its effect on infarct size reduction. This improvement could be explained by the increased capillary density and capillary-to-myocyte ratio, indicating formation of new blood vessels.

Acknowledgments

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