

*Full Paper***Effects of Erythropoietin on Cardiac Remodeling After Myocardial Infarction**

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Abstract. Erythropoietin (EPO) has been suggested to have a cardioprotective effect against ischemia. The purpose of this study was to examine the effects of EPO on cardiac remodeling after myocardial infarction (MI). MI was induced by ligation of the coronary artery in Wistar rats. The rats with MI were randomly divided into untreated MI and two EPO-treated MI groups. EPO was administered subcutaneously by injection once a day for 4 days after MI at 5000 U/kg or 3 times a week for 4 weeks at 1000 U/kg. Five days after MI, EPO prevented the increase in activated caspase 3, matrix metalloproteinase-2, and transcriptional activation of activator protein-1 in non-infarcted myocardium. Four weeks after MI, left ventricular weight, left ventricular end-diastolic pressure, and left ventricular dimension were increased, and ejection fraction and E wave deceleration time were decreased. EPO significantly attenuated this ventricular remodeling and systolic and diastolic dysfunction. In addition, EPO significantly attenuated the interstitial fibrosis and remodeling-related gene expression in non-infarcted myocardium. Furthermore, EPO significantly enhanced angiogenesis and reduced apoptotic cell death in peri-infarcted myocardium. In conclusion, when administered after MI, EPO prevents cardiac remodeling and improves ventricular function with enhanced angiogenesis and reduced apoptosis.

Keywords: myocardial infarction, cardiac remodeling, erythropoietin, angiogenesis, apoptosis

Introduction

Erythropoietin (EPO) is a glycoprotein hormone secreted from the kidney in response to decreased blood O₂ availability due to hypoxia and anemia (1). Since the primary function of EPO is to promote proliferation, differentiation, and survival of erythroid progenitors in the bone marrow, it increases production of red blood cells. Recombinant human EPO is therefore widely used for the treatment of anemia associated with chronic renal failure.

It has recently been found that EPO receptors are also

expressed by certain tissues outside of the hematopoietic system, such as endothelial cells, cardiomyocytes, and neurons (2–7). In addition, EPO has been shown to have protective effects against ischemic injury in the brain (8), spinal cord (9), retina (10, 11), kidney (12), and, most recently, even in myocardium (13–17). Current findings suggest that the protective effects of EPO in myocardial infarction (MI) depend on the anti-apoptotic effect of this cytokine. Recombinant human EPO reduced apoptosis and final infarct size in rats with MI, and these effects were accompanied by a reduction in decline of left ventricular contractile performance (13–15).

Because these cardioprotective effects have for the most part been investigated and observed in the acute phase of MI, the effects of EPO treatment on cardiac

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remodeling after MI are still unclear.

In the present study, we examined the effects of EPO treatment on activated caspase 3, matrix metalloproteinase-2 (MMP-2), and transcriptional activities of activator protein-1 (AP-1) in the subacute phase of MI and infarct size, cardiac remodeling, and systolic and diastolic function four weeks after MI, as well as myocardial fibrosis, apoptosis, angiogenesis, and expression of cardiac remodeling-related genes in non-infarcted myocardium.

Materials and Methods

Animals and experimental design

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 8523, revised 1996).

MI was induced in male Wistar rats weighing 290–310 g (Clea Japan, Osaka), as previously described (18). Briefly, the rats were anesthetized by injection of pentobarbital sodium (35 mg/kg, i.p.) and a left thoracotomy was performed under volume-controlled mechanical ventilation (tidal volume, 3.0 ml; respiratory rate, 60 cycles/min). Ligatures were then placed around the left anterior descending coronary arteries. Similar surgery was performed in sham-operated rats but without coronary artery ligation.

The rats with MI were randomly divided into untreated MI and EPO-treated MI groups. EPO was provided by Kirin Brewery Co., Ltd. (Osaka). EPO was administered subcutaneously by injection once a day for 4 days after MI at 5000 U/kg (EPO-H group, $n = 8$) or 3 times a week for 4 weeks at 1000 U/kg (EPO-L group, $n = 8$). The rats in the untreated MI group ($n = 8$) were administered vehicle (0.9% sodium chloride solution) in the same manner.

Western blot analysis

Protein extracts (20 μ g) from noninfarcted myocardium at five days after MI were separated on a 12% SDS-polyacrylamide gel and immobilized on polyvinylidene difluoride membrane. The membrane was immunoblotted with anti-phospho-ERK antibodies (Promega, Madison, WI, USA), anti-ERK antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), anti-phospho-p38 MAPK antibodies (New England Biolabs, Inc., Ipswich, MA, USA), anti-p38 MAPK antibodies (New England Biolabs, Inc.), anti-activated Caspase 3 (Abcam Ltd., Cambridge, UK) and MMP-2 (Daiichi Fine Chemical Co., Ltd., Toyama) using the ECL method for Western blot analysis. An optical scanner (EPSON GT-8000; Seiko, Tokyo) was used for

digitization of Western blots to allow for the measurement of phosphorylated proteins or of the total amount of protein. The densities of each band in digitized images of individual gels were measured using the public domain NIH Image program.

Electrophoretic mobility shift assay for AP-1

For the electrophoretic mobility shift assay, nuclear protein extracts at five days after MI were prepared as described previously (19). The sequence of the double-stranded oligonucleotide used in the present study was as follows: consensus AP-1, 5'-CGCTTGATGACTCAGCCGGAA-3'.

Doppler echocardiography and hemodynamic measurements

Four weeks after MI, we performed transthoracic echocardiography on each rat (20). The rats were lightly anesthetized with an injection of ketamine hydrochloride (25–50 mg/kg, i.p.) and xylazine (5–10 mg/kg, i.p.). Echocardiograms were performed with an echocardiography system equipped with a 7.5-MHz phased-array transducer (SONOS 5500; Philips Medical System, Best, The Netherlands). Two-dimensional short-axis view of the left ventricle and M-mode tracings were recorded through the anterior and posterior left ventricular (LV) walls at the papillary muscle level to measure LV end-diastolic dimension (LVDd) and LV end-systolic dimension (LVDs). LV ejection fraction (EF) was measured by the modified Simpson's method, which used a 4-chamber view. Pulsed-wave Doppler spectra of mitral inflow were recorded from the apical 4-chamber view, with the sample volume placed near the tips of the mitral leaflets in maximal opening with laminar flow pattern.

The method of hemodynamic measurement used was previously described in detail (18). In brief, LV pressure was recorded by inserting a polyethylene-tubing catheter (0.58-mm internal diameter, PE-50) into the right carotid artery and advancing it into the left ventricle. Water-filled catheters were connected to the tubing, which was in turn connected to a water-filled pressure transducer. The pressures were recorded on a physiological recorder, while rats were allowed to breathe spontaneously. LV end-diastolic pressure (LVEDP) was obtained by averaging the values for 10 beats. Myocardial infarct size was measured as previously described (18). Rats with an infarct size of <10% were excluded from analysis. After determination of infarct size, the heart was immediately excised and septal myocardium was dissected as non-infarcted myocardium.

Histological examination

Transverse myocardial sections (5- μ m-thick) obtained four weeks after MI were stained with collagen-specific sirius red stain. Each field of non-infarcted myocardium was digitized and the area of interstitial fibrosis was calculated as the ratio of the sum of total area of interstitial fibrosis to the sum of total connective tissue area and cardiomyocyte area in all LV fields of the section. Perivascular areas were not included in this analysis.

TUNEL assay was performed using the ApopTag Apoptosis Detection Kit (Intergen, Burlington, MA, USA) followed by methyl green counterstaining of formalin-fixed, paraffin-embedded sections. Digital photographs were taken under microscopy at $\times 400$ magnification. Twenty-five random high-power fields in the peri-infarct zone of each ventricular sample were chosen and blindly quantified.

Immunohistochemical staining for CD31 (DAKO, Kyoto) and vascular density analysis were performed. After excision, the heart was immediately embedded in OCT compound (Tissue Tek; Miles, Inc., Elkhart, IN, USA), frozen in dry ice/acetone, and cut into 5- μ m sections using a cryostat. Five high-power fields in each section were randomly selected in the peri-infarct area, and the number of capillaries in each field was averaged and expressed as the number of capillary vessels per high-power field.

RNA preparation and Northern blot analysis

All procedures were performed as previously described in detail (20). In brief, total RNA was isolated from each heart by the guanidium thiocyanate-phenol-chloroform method. Twenty micrograms total RNA samples were subjected to 1% agarose gel electro-

phoresis, transferred to nylon membrane, and hybridization was carried out with (32 P)-dCTP-labeled cDNA probes for brain natriuretic peptide (BNP), collagen types I and III, osteopontin, plasminogen activator inhibitor-1 (PAI-1), monocyte chemoattractant protein-1 (MCP-1), and rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The densities of individual mRNA bands were measured using a bioimaging analyzer (BAS-2000; Fuji Photo Film, Tokyo). For all RNA samples, the density of an individual mRNA band was divided by that of the GAPDH mRNA band to correct for differences in RNA loading and/or transfer.

Statistical analyses

All results are expressed as the mean \pm S.E.M. The statistical significance of differences was determined using ANOVA and the Student-Newman-Keuls test. Statistical significance was assumed at $P < 0.05$.

Results

Western blot analysis

To analyze the effects of EPO on molecular mechanisms, Western blot analysis was performed from the sample of the non-infarcted myocardium five days after MI. As shown in Fig. 1, EPO did not change the activities of myocardial ERK and p38 MAPK. The activated caspase-3 in the non-infarcted myocardium was significantly increased in the untreated MI group (5.1-fold, $P < 0.01$). EPO treatment significantly attenuated the activated caspase-3 compared with the untreated MI group (EPO-L: 66%, $P < 0.01$; EPO-H: 60%, $P < 0.01$). MMP-2 was significantly increased in the untreated MI group (2-fold, $P < 0.01$). EPO significantly attenuated the increase of MMP-2 in the non-infarcted

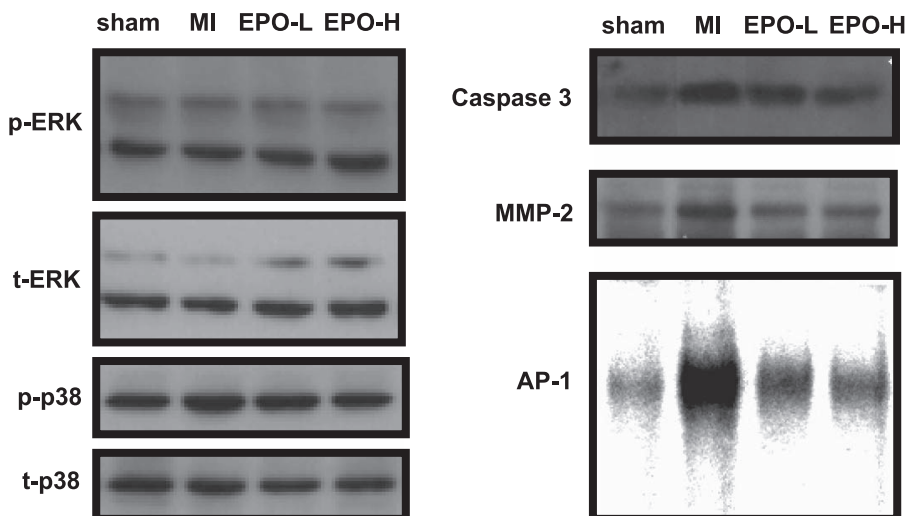


Fig. 1. Effects of EPO on non-infarcted myocardial ERK, p38 MAPK, activated caspase-3, MMP-2, and AP-1 DNA binding activity at five days after MI. The left panels, right top, and right middle panels show representative autoradiographs of Western blot analysis of phosphorylated ERK (p-ERK), total amount of ERK (t-ERK), phosphorylated p38 MAPK (p-p38), total amount of p38 MAPK (t-p38), activated Caspase-3, and MMP-2. The right lower panel shows a representative autoradiograph of gel mobility shift assay of AP-1 DNA binding activity.

myocardium, compared with the untreated MI group (EPO-L: 52%, $P<0.01$; EPO-H: 54%, $P<0.01$).

Electrophoretic mobility shift assay for AP-1

As shown in Fig. 1, MI significantly increased the myocardial transcriptional activity of AP-1 2.5-fold in the non-infarcted myocardium ($P<0.01$). EPO significantly inhibited the increase in the DNA binding activities of AP-1 (EPO-L: 61%, $P<0.01$; EPO-H: 56%, $P<0.01$).

Effects of EPO on ventricular weight, hemodynamics, and infarct size

As shown in Table 1, MI significantly reduced mean blood pressure ($P<0.05$) and increased LVEDP ($P<0.01$) and the LV weight/body weight ratio ($P<0.01$). EPO treatment affected neither heart rate nor mean blood pressure. However, both low and high doses of EPO significantly prevented the increase in weight of the left ventricle (EPO-L, $P<0.05$ and EPO-H, $P<0.01$) and reduced LVEDP (EPO-L, $P<0.01$ and EPO-H, $P<0.05$), compared with rats with untreated MI. Infarct size calculated as fractional area of the scar was significantly smaller in both the EPO-L and EPO-H groups than in the MI group.

Echocardiographic assessment of LV

As shown in Table 1 and Fig. 2, the LVDd and LVDs were significantly higher in the MI group than in the sham-operated group ($P<0.01$ and $P<0.01$, respec-

tively). EPO prevented the increases in both LVDd and LVDs compared with the untreated MI group (EPO-L, $P<0.05$ and $P<0.05$; EPO-H, $P<0.05$ and $P<0.05$, respectively). The MI group exhibited significant systolic dysfunction compared with the sham-operated group, as evidenced by decreased EF ($P<0.01$). Both doses of EPO significantly prevented the decrease in EF (EPO-L, $P<0.01$ and EPO-H, $P<0.05$).

A parameter of diastolic dysfunction, the deceleration time of the E wave was shorter in the untreated MI group than in the sham-operated group ($P<0.01$). EPO significantly prevented worsening of diastolic function after MI in both the EPO-L and EPO-H groups (EPO-L, $P<0.01$ and EPO-H, $P<0.01$). The ratio of E wave to A wave (E/A ratio) in the untreated MI group was significantly increased compared with the sham-operated group ($P<0.05$). In the EPO-L group, E/A ratio was significantly improved compared with the untreated MI group ($P<0.05$). E/A in the EPO-H group did not change compared with the untreated MI group.

Histological and morphometric assessments

As shown in Fig. 3A, sirius red staining revealed a 5.7-fold increase in the fraction of interstitial fibrosis in non-infarcted myocardium in rats with untreated MI compared with sham-operated rats ($P<0.01$). EPO treatment significantly reduced the fraction of interstitial fibrosis (EPO-L: 0.25-fold, $P<0.01$; EPO-H: 0.34-fold, $P<0.01$).

Figure 3B shows enhanced angiogenesis, with a larger

Table 1. Hemodynamics, ventricular weight, and Doppler echocardiographic measurements

	sham	MI		
		untreated	EPO 1000 U/kg	EPO 5000 U/kg
Heart rate (bpm)	319 ± 26	303 ± 31	326 ± 28	319 ± 27
MBP (mmHg)	105 ± 2	93 ± 2*	95 ± 3*	93 ± 5*
LVEDP (mmHg)	9 ± 1	16 ± 2**	10 ± 1 ^{§§}	12 ± 2 [§]
Body weight (g)	355 ± 6	344 ± 4.7	326 ± 4.6** [§]	330 ± 4.6**
LV/BW (g/kg)	2.09 ± 0.05	2.349 ± 0.03**	2.22 ± 0.049 [§]	2.143 ± 0.042 ^{§§}
RV/BW (g/kg)	0.546 ± 0.007	0.657 ± 0.03	0.621 ± 0.018	0.781 ± 0.08**
MI size (%)	—	38.8 ± 2.3	20.7 ± 5.2 [§]	26 ± 4.4 [§]
LVDd (mm)	7.9 ± 0.2	9.9 ± 0.4**	8.6 ± 0.5 [§]	8.9 ± 0.3 [§]
LVDs (mm)	5.8 ± 0.2	8.6 ± 0.3**	7.0 ± 0.6* [§]	7.4 ± 0.4* [§]
EF (%)	59.4 ± 1.1	29 ± 3.6**	45 ± 4.3** ^{§§}	38 ± 2.7** ^{§§}
E wave deceleration time (ms)	56.9 ± 1.8	39.1 ± 2.2**	56.6 ± 2.6 ^{§§}	52 ± 1.8 ^{§§}
E/A	1.7 ± 0.2	3.8 ± 0.7*	1.7 ± 0.2 [§]	3.1 ± 0.2*

sham: sham operated rats, MI: myocardial infarction, MBP: mean blood pressure, LVEDP: left ventricular end-diastolic pressure, LV/BW: left ventricle weight/body weight, RV/BW: right ventricle weight/body weight, LVDd: left ventricular dimension end diastole, LVDs: left ventricular dimension end systole, EF: ejection fraction. * $P<0.05$, compared with sham; ** $P<0.01$, compared with sham; [§] $P<0.05$, compared with untreated MI; ^{§§} $P<0.01$, compared with untreated MI.

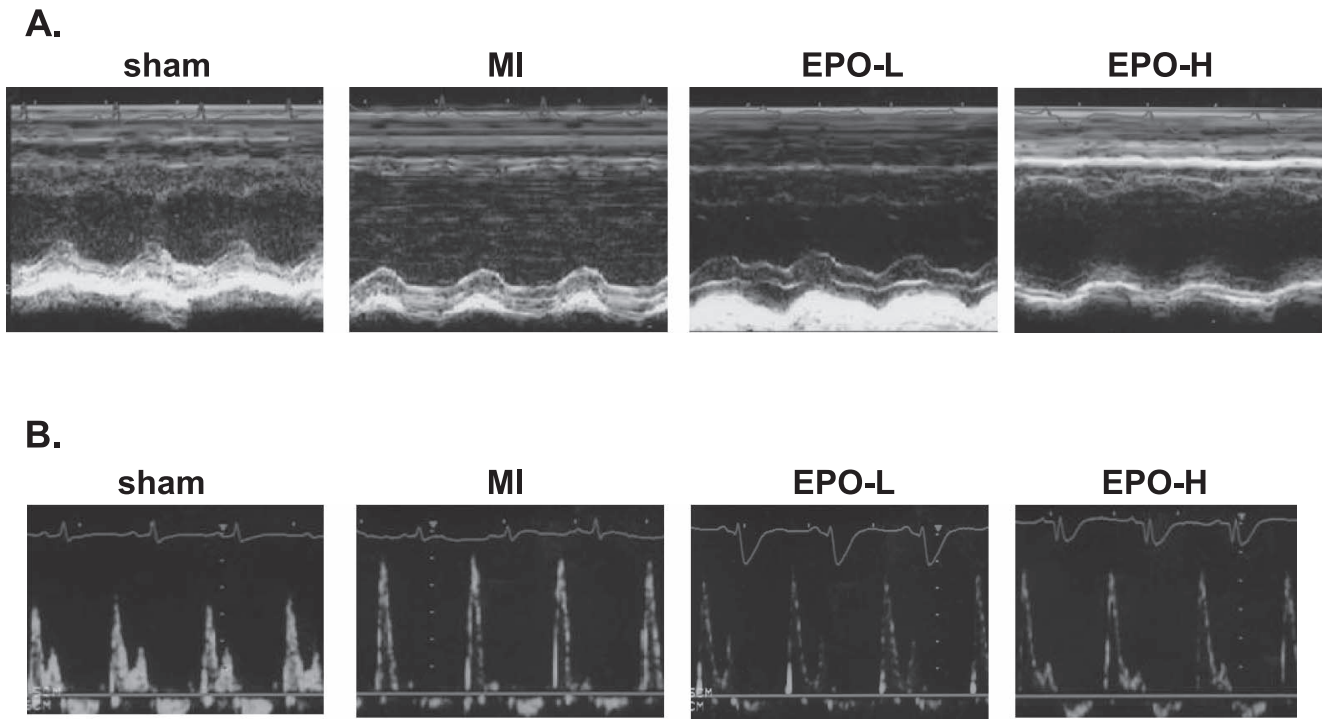


Fig. 2. Echocardiographic assessment 4 weeks after MI. A: LV M-mode echocardiograms (parasternal short-axis view) from a sham-operated rat (sham), a rat with untreated MI (MI), and infarcted rats treated with EPO (EPO-L and EPO-H). B: Examples of pulse-wave Doppler spectra of mitral inflow from a sham-operated rat (sham), a rat with untreated MI (MI), and infarcted rats treated with EPO (EPO-L and EPO-H).

number of capillaries, in the EPO treatment groups (EPO-L: 8.4 ± 0.8 capillaries/HPF, $P < 0.01$ to MI; EPO-H: 5.5 ± 0.3 capillaries/HPF, $P < 0.05$ to MI; MI: 3.1 ± 0.5 capillaries/HPF). Moreover, EPO-L treatment induced formation of significantly more capillaries than did EPO-H treatment ($P < 0.01$).

Figure 3C illustrates TUNEL staining of peri-infarcted myocardium at four weeks after MI. More apoptotic nuclei were observed in untreated MI. Each of the EPO-treated groups exhibited significantly fewer TUNEL-positive nuclei than the untreated MI group (EPO-L: 4.0 ± 0.5 cells/HPF, $P < 0.05$ to MI; EPO-H: 5.1 ± 1.1 cells/HPF, $P < 0.05$ to MI; MI: 8.5 ± 1.5 cells/HPF).

mRNA expression in non-infarcted myocardium

Figure 4 shows the results of analysis of cardiac gene expression. mRNA expressions of BNP, collagen types I and III, osteopontin, PAI-1, and MCP-1 were significantly increased 4.5-, 4.8-, 3.2-, 5.1-, 3.4-, and 2.2-fold, respectively, at four weeks after MI ($P < 0.01$) in non-infarcted myocardium. EPO significantly attenuated the increases in expression of BNP (40%, $P < 0.05$), collagen I (58%, $P < 0.05$), collagen III (43%, $P < 0.05$), osteopontin (43%, $P < 0.05$), PAI-1 (38%, $P < 0.05$), and

MCP-1 (50%, $P < 0.05$) mRNA in the EPO-L group. In the EPO-H group, EPO significantly attenuated the increases in expression of BNP (44%, $P < 0.05$), collagen I (62%, $P < 0.05$), collagen III (63%, $P < 0.05$), osteopontin (49%, $P < 0.05$), PAI-1 (59%, $P < 0.05$), and MCP-1 (45%, $P < 0.05$) mRNA.

Discussion

In the present study, we have shown that EPO treatment after MI reduces infarct size, prevents cardiac remodeling, and improves LV function, and also enhances angiogenesis and inhibits myocardial apoptosis. Like short-term treatment with high doses of EPO, long-term treatment with low doses of EPO may thus be an effective strategy for treatment of MI.

We and other investigators have previously reported the preventive effects of angiotensin-converting enzyme (ACE) inhibitor, angiotensin II type 1 receptor blocker (ARB), and other such neurohumoral factor blockers on cardiac remodeling after MI (19–21). However, the anti-cardiac remodeling effects of these cardioprotective agents were observed without reduction of infarct size after MI. Notably, though, reduction of infarct size may be one of the main mechanisms by which EPO

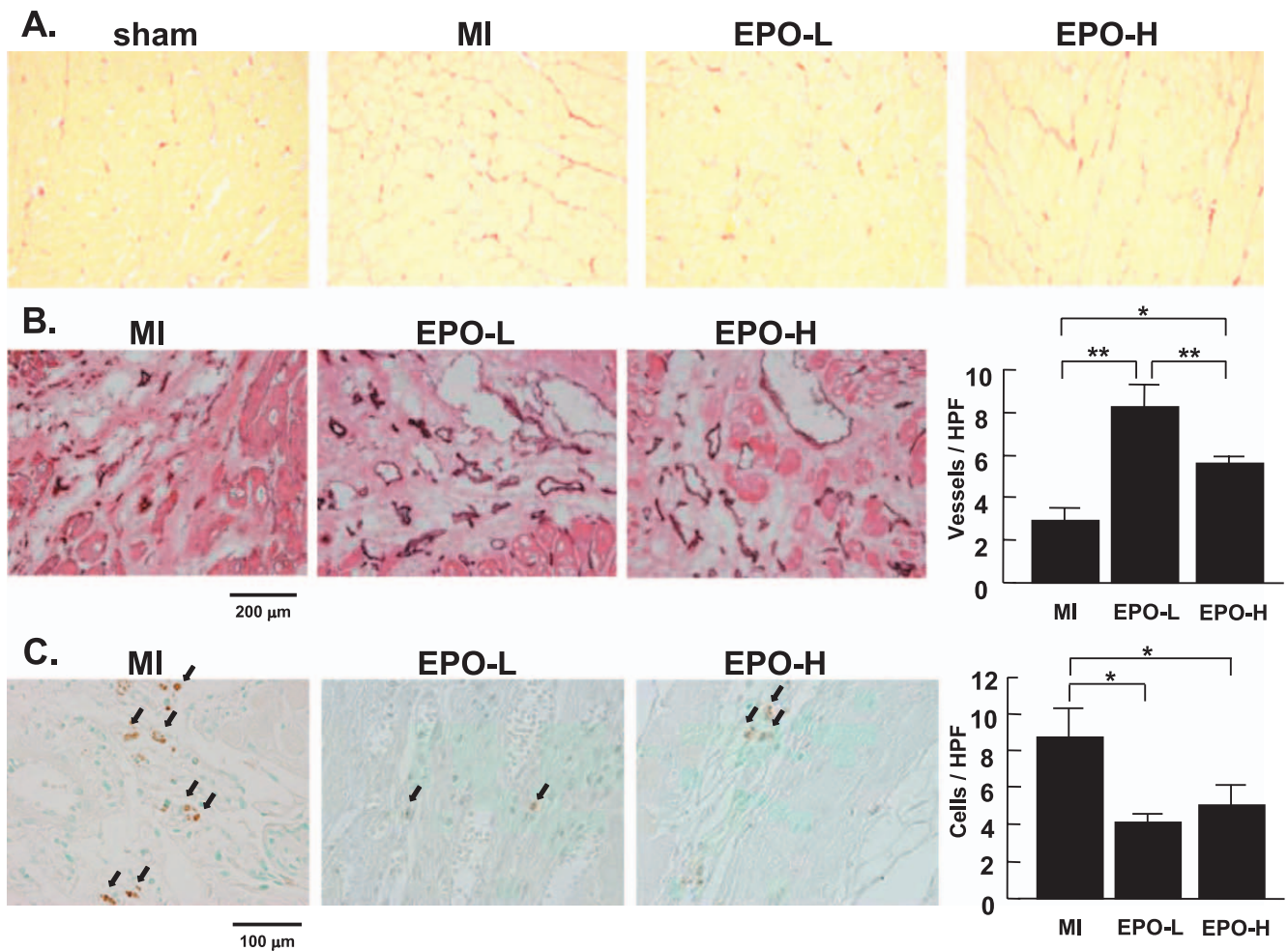


Fig. 3. Histopathological analysis of non-infarcted myocardium of rat left ventricle. A: Photomicrographs show sirius red-stained cardiac sections from sham-operated rats (sham), rats with untreated MI (MI), and infarcted rats treated with EPO (EPO-L and EPO-H). B: Capillary density of peri-infarct region 4 weeks after MI. Sections show immunohistochemical staining for CD31. Capillary density is expressed as the number of capillary vessels per high-power field. Values are each a mean \pm S.E.M. * P <0.05, ** P <0.01. C: Number of TUNEL-positive cells in the peri-infarct region 4 weeks after MI. Values are each a mean \pm S.E.M. * P <0.05.

attenuates cardiac remodeling after MI. It has previously been reported that EPO administered at various time points exerted marked protective effects against ischemia/reperfusion injury and reduced infarct size in rats and rabbits (13 – 17, 22). One of the mechanisms by which EPO may be able to reduce infarct size after MI is anti-apoptotic effects (13 – 15). Also in other organs, EPO exerts protective effects through the inhibition of apoptosis (10 – 12). Activation of Akt and/or PI3 kinase and inhibition of apoptosis are reported to be associated with infarct size-limiting effects of EPO (14). In the present study, activated caspase-3 in the non-infarcted myocardium was reduced in the EPO-treated groups in the subacute phase of MI. Thereafter, the EPO-treated groups exhibited significantly fewer TUNEL-positive nuclei in the peri-infarct area than the untreated MI

group. The mean infarct sizes in the EPO-treated MI groups were significantly lower than that in untreated MI. Our findings and those noted in previous reports thus suggest that administration of EPO after MI prevented LV remodeling and reduced infarct size, which may be partially due to inhibition of myocardial apoptosis.

Furthermore, cardiomyocyte apoptosis is not limited to the acute stages of MI but remains increased in the viable myocardium adjacent to infarction even until several weeks after experimental MI (23). Late after MI, apoptosis in the non-infarcted myocardium may play a role in post-MI ventricular remodeling and thus may contribute to the development of congestive heart failure (23). The reduction of apoptosis in the both peri-infarct and non-infarcted myocardium by EPO treatment may

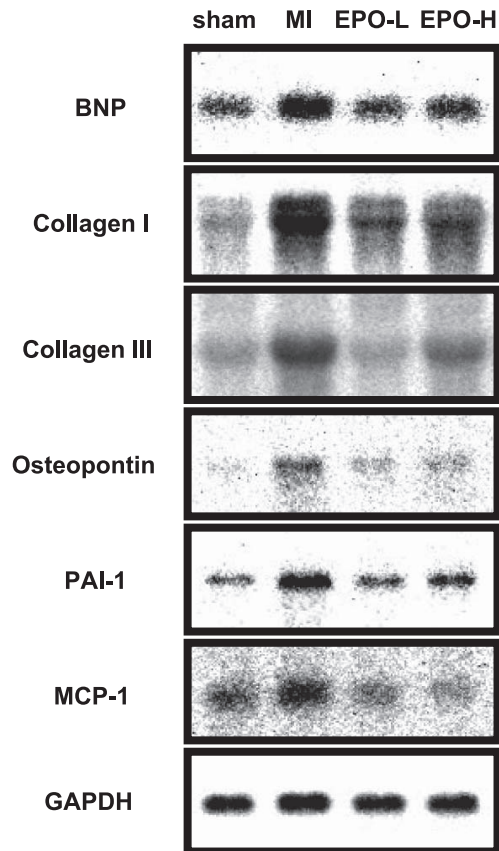


Fig. 4. Effects of EPO on non-infarcted myocardial mRNA expression 4 weeks after MI. Myocardial mRNA expressions of brain natriuretic peptide (BNP), collagen types I and III, osteopontin, plasminogen activator inhibitor 1 (PAI-1), and monocyte chemo-attractant protein 1 (MCP-1) were compared in sham-operated rats (sham), rats with untreated MI (MI), and infarcted rats treated with EPO (EPO-L and EPO-H). Each panel shows a representative autoradiogram of Northern blot analysis of BNP, collagen I, collagen III, osteopontin, PAI-1, and MCP-1.

be correlated with the improvement of LV remodeling and the prevention of heart failure.

A significant proportion of the delayed form of myocardial damage occurs as a result of recruitment of inflammatory cells into not only the infarcted region but also non-infarcted regions, in which chemotactic and cytotoxic cytokines and other inflammatory molecules induce cardiomyocyte hypertrophy and myocardial fibrosis and expand the volume of infarct region in an amplifying positive-feedback loop. Previous studies reported anti-inflammatory and cytoprotective effects of EPO (24). In the present study, we measured the DNA binding activity of AP-1 as a key transcriptional factor for cell regulation in a non-infarcted region during cardiac remodeling after MI as we previously reported (19). EPO significantly attenuated the increased activation of non-infarct myocardial AP-1 after MI, thereby

suggesting the effects of EPO on transcriptional activation for cardiac remodeling.

Inflammatory cells, which are increased in very early and early stages, produce MMPs that modulate extracellular collagen matrix remodeling. We evaluated the amount of MMP-2 in the non-infarcted myocardium five days after MI. In the EPO-treated groups, MMP-2 was significantly reduced compared with the untreated MI group, thereby suggesting the anti-inflammatory effects of EPO.

In the present study, mRNA expressions of collagen types I and III, osteopontin, PAI-1, and MCP-1 in non-infarcted myocardium were significantly decreased by EPO treatment. We also found that EPO significantly prevented the increase in cardiac fibrosis. Although we did not find significant difference between the expressions of cardiac remodeling-related genes in the EPO-L group and the EPO-H group, the EPO-L group exhibited a trend towards lower expression of these genes. Furthermore, heart failure in the EPO-L group seemed to be more improved with lower expression of BNP than in the EPO-H group. The amount of collagen increased in the non-infarcted myocardium mainly during the late stage after MI, so the EPO treatment during this period may decrease fibrosis and prevent dysfunction much more than short-term EPO treatment.

Another beneficial effect of EPO, enhancement of angiogenesis in the peri-infarct area, may contribute to prevention of cardiac remodeling after MI. It has been shown that EPO induces angiogenesis in a rat aortic ring model (25) and in the chick chorioallantoic membrane (26). It has also been reported that EPO stimulates capillary outgrowth in an *in vitro* angiogenesis assay using adult human myocardial tissue (27). In the present study, enhancement of angiogenesis with increase in number of capillaries was observed in the EPO treatment groups. The increase of capillary in the peri-infarct area can improve inadequate oxygenation and nutrient supply after MI and prevent apoptosis, reduce progressive collagen deposition and scar formation, and improve ventricular function. Moreover, long-term treatment with EPO induced formation of significantly more capillaries than did short-term treatment. The constant increase of capillary in the peri-infarct area with the long-term EPO treatment can provide adequate oxygenation and nutrient supply and go on preventing progressive cardiac remodeling late after MI. These findings suggest that the neovascularization induced by EPO may play an important role in preventing cardiac remodeling. Long-term treatment with EPO may be particularly effective for enhancement of angiogenesis in the peri-infarct area and can prevent progressive cardiac remodeling late after MI. In the present study, although

both EPO treatment groups exhibited attenuation of LV dilatation and improved LV systolic function after MI, the EPO-L group exhibited a trend towards smaller LVDD and LVDs, lower EDP, larger EF, and lower expression of BNP, compared with the EPO-H group. These anti-remodeling effects of EPO-L may result from the angiogenesis induced by EPO.

In the previous studies, several doses of EPO (1000 – 5000 U/kg) as a single administration significantly reduced myocardial infarction (13 – 17). Parsa et al. found that the infarct size in the rabbits treated with a single dose of 1000 U/kg was equally reduced and similar to that in those with 5000 U/kg (16). In the present study, we did not find any significant difference between the infarct size of EPO-L group and EPO-H group. In the present study, we also showed that a lower dose of EPO may be effective for treatment of MI.

In conclusion, we have demonstrated that EPO can reduce MI size and prevent cardiac remodeling and cardiac dysfunction after MI. Long-term treatment with low doses of EPO after MI increases capillary number to a greater extent than does short-term treatment. The expression of cardiac remodeling-related genes is likely to be lower after MI with long-term EPO treatment. Thus, long-term treatment for MI with low doses of EPO may be an effective therapeutic strategy for angiogenesis and prevention of cardiac remodeling.

Acknowledgments

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