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Invited Review

Current Perspectives on Protective Roles of Erythropoietin in Cardiovascular System: Erythropoietin Receptor as a Novel Therapeutic Target

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Erythropoietin (EPO) is a principal regulator that promotes proliferation and terminal differentiation of erythroid progenitor cells. EPO receptors are expressed not only in hematopoietic lineage cells but also in the cardiovascular system. We performed animal experiments using transgene-rescued EPO receptor null mutant mice ($EpoR^{-/-}_{rescued}$) that express the EPO receptor exclusively in the hematopoietic cells. The results of these experiments suggest that endogenous EPO/EPO receptor system in the heart exerts cardioprotective effects against myocardial injury induced by ischemia followed by reperfusion and pressure-overload induced left ventricular dysfunction. Many animal experiments have shown that the administration of recombinant human EPO also elicits cardioprotective effects against myocardial injury induced by ischemia and reperfusion. In contrast to the promising results of these animal experiments, recent clinical trials failed to demonstrate the reduction in infarct size or improvement of cardiac function by the administration of recombinant human EPO in patients with acute myocardial infarction who underwent primary percutaneous coronary intervention. It should be tested in future clinical studies whether a relatively low dose of recombinant human EPO or its derivatives that have no erythropoietic action reduces infarct size and ameliorates cardiac dysfunction in patients with acute myocardial infarction. In this article, we review implications of anemia associated with chronic heart failure, roles of the endogenous EPO/EPO receptor system, and the effects of the administration of erythropoiesis-stimulating agents in pathologic conditions of the heart by focusing on the EPO receptor as a potential candidate of novel therapeutic targets in cardiovascular diseases.

Keywords: anemia; erythropoietin; left ventricular hypertrophy; myocardial infarction; ventricular remodeling
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Erythropoietin (EPO) is a cytokine that is produced in the adult kidney and fetal liver in mammals. EPO was originally discovered as a principal regulator that promotes proliferation and terminal differentiation of erythroid progenitor cells by preventing apoptosis (Koury and Bondurant 1990). There is a high degree of sequence homology in the coding region of EPOs among mammalian species (Shoemaker and Mitsock 1986; Wen et al. 1993). EPO receptors are expressed not only in hematopoietic lineage cells but also in nonhematopoietic organs (Digicaylioglu et al. 1995; Grimm et al. 2002), including the heart (Calvillo et al. 2003; Parsa et al. 2003; Wright et al. 2004). Roles of EPO in nonhematopoietic cells include the promotion of endothelial cell proliferation (Anagnostou et al. 1994) and the amelioration of neuronal recovery from injury (Sakanaka et al. 1998; Siren et al. 2001). EPO signaling in nonhematopoietic cells might be transduced by a receptor that is distinct from that expressed in hematopoietic cells (Vogel and Gassmann 2011). The EPO receptor in hematopoietic cells functions as a monodimer (Watowich et al. 1994). However, the EPO receptor in nonhematopoietic cells has been speculated to be a heterodimer with one EPO receptor monomer and the common βc-subunit that is shared by the receptors for interleukin 3, interleukin 5 and granulocyte macrophage colony-stimulating factor (Brines et al. 2004), although further studies are still needed to confirm a diversity of the EPO receptor.

We have focused on dissecting roles of endogenous

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EPO/EPO receptor system in cardiovascular diseases in a clinical study (Namiuchi et al. 2005) and animal experiments using transgene-rescued EPO-receptor null mutant mice ($EpoR^{-/-}{}_{\text{rescued}}$) that express the EPO receptor exclusively in the hematopoietic cells (Suzuki et al. 2002). The results of these experiments suggest that endogenous EPO/EPO receptor system in the cardiovascular system plays important protective roles in both humans and murine models of various cardiovascular diseases (Satoh et al. 2006; Tada et al. 2006; Asaumi et al. 2007; Nakano et al. 2007). Recent studies by others have demonstrated that the administration of recombinant human EPO elicits cardioprotective effects against myocardial injury induced by ischemia and reperfusion (Tramontano et al. 2003; Calvillo et al. 2003; Moon et al. 2003; Cai et al. 2003; Parsa et al. 2003; Wright et al. 2004; Najjar et al. 2011). In contrast to the promising results of these animal experiments, recent clinical trials demonstrated that the administration of erythropoiesis stimulating agents, such as recombinant human EPO and the derivative of EPO, failed to reduce the size of myocardial infarction or to improve cardiac function in patients with acute myocardial infarction who underwent successful primary percutaneous coronary intervention (Lipsic et al. 2006; Ott et al. 2010; Voors et al. 2010; Najjar et al. 2011). In this article, we review implication of anemia associated with chronic heart failure, roles of endogenous EPO/EPO receptor system in cardiovascular diseases, and effects of the administration of erythropoiesis stimulating agents in myocardial ischemia and reperfusion focusing on the EPO receptor as a potential candidate of novel therapeutic targets in cardiovascular diseases.

EPO in anemia associated with chronic heart failure

Anemia is one of the independent predictors of unfavorable outcomes in patients with chronic heart failure. Go et al. (2006) examined the associations between hemoglobin levels, kidney function, and the risks of death and hospitalization in 59,772 patients with chronic heart failure, and found that reduced (< 13 g/dL) hemoglobin levels and chronic kidney disease independently predict increased risks of death and hospitalization due to worsening of heart failure, regardless of the level of left ventricular systolic function. We prospectively studied 357 stable patients with chronic heart failure and preserved left ventricular systolic function (diastolic heart failure) for a mean follow-up period of 3.6 years, and found that lower hemoglobin levels were significantly associated with the development of sudden death using a multivariate model that included the estimated glomerular filtration rate (Tada et al. 2008). Although patients with chronic heart failure are frequently associated with both chronic kidney disease and anemia (McClellan et al. 2002), the results of these clinical studies strongly suggest that anemia independently predicts cardiac events in patients with chronic heart failure regardless of the severity of chronic kidney disease and left ventricular dysfunction. Possible mechanisms include exacerbated peripheral and myocardial tissue hypoxia, increased levels of proinflammatory cytokines, accelerated progression of left ventricular hypertrophy and dilatation, and activation of the sympathetic nervous system and renin-angiotensin-aldosterone system (Go et al. 2006).

Underlying causes of anemia in patients with chronic heart failure comprise decreased EPO secretion and impaired bone marrow response to EPO (Tang and Katz 2006). Plasma levels of proinflammatory cytokines such as tumor necrosis factor-α and interleukin-6 are increased in patients with chronic heart failure in a severity-dependent manner (Torre-Amione et al. 1996). These proinflammatory cytokines have been shown to impair erythropoiesis by reducing the secretion of EPO from the kidney, supressing the activity of EPO in the bone marrow, and reducing bioavailability of iron (Weiss and Goodnough 2005). Angiotensin II stimulates erythropoiesis by accelerating the expression of EPO in the kidney (Katz 2004) and stimulating the proliferation of erythroid progenitor cells (Mrug et al. 1997). Inhibition of renin-angiotensin system by angiotensin coverting enzyme inhibitors or type 1 angiotensin II receptor blockers, both of which are essential for the treatment of patients with chronic heart failure due to left venticular systolic dysfunction (The SOLVD investigators. 1991; Pfeffer et al. 2003), can be also one of the causes of impaired erythropoiesis in patients with chronic heart failure (Tang and Katz 2006). The results of these ealier studies strongly suggest that EPO plays a key role in the pathogenesis of anemia that is frequently associated with chronic heart failure.

In a small clinical study, a significant improvement in cardiac and patient function was reported when patients with mild anemia and moderate to severe chronic heart failure due to reduced left ventricular systolic function were treated with recombinant human EPO and intravenous iron (Silverberg et al. 2001). In this study, the mean hemoglobin levels were 10.9 and 10.3 g/dL before the treatment and 10.8 and 12.9 g/dL after the treatment in the control and treated groups, respectively. The left ventricular ejection fraction was decreased by 5.4% in the control group and increased by 5.5% in the treated gourp. The mean New Yourk Heart Association functional class worsened by 11.4% in the control group and improved by 42.1% in the treated group. Mancini et al. (2003) investigated the effects of recombinant human EPO on exercise capacity in 26 patients with anemia and moderate to severe chronic heart failure. They found that there were significant increases in hemoglobin levels (from 11.0 to 14.3 g/dL), peak oxygen consumption during exercise, and exercise duration in the treated group but no changes in the control group. In 1,233 patients with clinical evidence of congestive heart failure or ischemic heart disease who were receiving hemodialysis, however, Besarab et al. (1998) demonstrated that normalizing hematocrit (42%) by recombinant human EPO was associated with a trend toward a higer event rate of death or nonfatal acute myocardioal infarction as compared with tar-

geting a lower hematocrit level (30%). The results of these clinical studies suggest that effectiveness of recombinant human EPO in patients with anemia and heart dieases may depend on both commorbidity burden of the patients and target hemoglobin levels that are achieved by the administration of EPO. Importance of the target hemoglobin level is also confirmed in antother two large clinical trials in which recombinant human EPO was administered to patients with chronic kidney disease and anemia (Drüeke et al. 2006; Singh et al. 2006). In these trials, normal and subnormal hemoglobin targets achieved by the treatment with recombinant human EPO were compared in patients with chronic kidney disease and anemia. The investigators of these two large clinical studies demonstrated that normalizing hemoglobin levels was associated with increased risks of cardiovascular events, the progression of chronic kidney disease, and poor quality of life as compared with targeting a lower hemoglobin level in the patients with chronic kidney disease and anemia. Although precise mechanisms of the unfavorable outcomes brought by normalizing hemoglobin levels using recombinant human EPO remain to be elucidated, the results of these studies suggest that we have to consider carefully possible adverse outcomes due to accelerated erythropoiesis when we treat patients with anemia associated with chronic heart failure or chronic kidney disease using EPO.

Protective effects of endogenous EPO/EPO receptor system in cardiovascular diseases

Epo mRNA and protein have been shown to be expressed not only in the kidney but also in the testis, liver, central nervous system and the female reproductive organs (Sasaki et al. 2001). EPO receptors also have been demonstrated to be expressed not only in hematopoietic cells but also in other nonhematopoietic cells and organs such as endothelial cells (Anagnostou et al. 1994), the brain including the hippocaupus, capsula interna, cortex, and midbrain (Digicaylioglu et al. 1995), and photoreceptor cells in the retina (Grimm et al. 2002). More recent studies have shown that the EPO receptor is expressed in cardiomyocytes (Cai et al. 2003; Tramontano et al. 2003; Wright et al. 2004). These results strongly suggest that circulating EPO interacts with the EPO receptor that is expressed in nonhematopoietic cells. However, it was difficulat to differentiate direct effects of EPO on nonhematopoietic cells or organs that express the EPO receptor from indirect effects of EPO through erythropoiesis. In this regard, Suzuki et al. (2002) have developed a very unique animal model. EPO receptor null mice die by embriogenic day 13.5 due to severe anemia (Lin 1996). Using a *GATA-1* minigene cassette with hematopoietic regulatory domains, Suzuki et al. (2002) developed transgene-rescued EPO receptor null mutant mice ($EpoR^{-/-}{}_{\text{rescued}}$) that express the EPO receptor exclusively in the hematopoietic cells. $EpoR^{-/-}{}_{\text{rescued}}$ mice do not have anemia, develop normally and are fertile even though they are totally deficient in the EPO receptor in the nonhematopoietic cells including those in the cardiovascular system.

Using $EpoR^{-/-}{}_{\text{rescued}}$ mice, we have demonstrated that endogenous EPO/EPO receptor system in nonhematopoietic cells plays an important protective role in various pathologic conditions in the cardiovascular system. We reported that infarct size after 30-minute left coronary artery ligation followed by 24-hour reperfusion was increased in $EpoR^{-/-}{}_{\text{rescued}}$ mice as compared with wild-type mice (Tada et al. 2006). Phosphorylation of p38 and that of JNK in the ischemic area were increased in wild-type mice, but not in $EpoR^{-/-}{}_{\text{rescued}}$ mice as compared with corresponding sham-operated mice. Caspase-3 activity and number of TUNEL-positive cardiomyocytes in the ischemic area were increased in $EpoR^{-/-}{}_{\text{rescued}}$ mice as compared with wild-type mice. These results suggest that endogenous EPO/EPO receptor system in nonhematopoietic lineage cells plays an important protective role against the myocardial injury induced by ischemia followed by reperfusion at least in part by preventing apoptosis (Fig. 1). We then investigated whether the endogenous EPO/EPO receptor system in nonhematopoietic cells also plays a protective role against pressure overload-induced cardiac dysfunction (Asaumi et al. 2007). We found that left ventricular end-diastolic diameter was significantly increased, left ventricular fractional shortening was significantly decreased, and survival rate was significantly decreased in $EpoR^{-/-}{}_{\text{rescued}}$ mice with 1-week transverse aortic constriction as compared with wild-type mice with transverse aortic constriction. Phosphorylation of STAT3 and that of p38 were increased in wild-type mice after transverse aortic constriction, but not in $EpoR^{-/-}{}_{\text{rescued}}$ mice as compared with corresponding sham-operated mice. Vascular endothelial growth factor protein expression and capillary density in left ventricular myocardium were significantly increased in the wild-type mice with transverse aortic constriction, but not in the $EpoR^{-/-}{}_{\text{rescued}}$ mice with transverse aortic constriction, as compared with corresponding sham-operated mice. These data suggest that the endogenous EPO-EPO receptor system in nonhematopoietic cells also plays an important protective role against pressure-overload induced cardiac dysfunction at least in part by coronary angiogenesis (Fig. 1).

The importance of protective roles of endogenous EPO-EPO receptor system has been demonstrated in murine models of vascular diseases as well. Using $EpoR^{-/-}{}_{\text{rescued}}$ mice, we demonstrated that endogenous EPO-EPO receptor system contributed to the mobilization of endothelial progenitor cells, their recruitment to the pulmonary artery, and the prevention of the development of hypoxia-induced pulmonary hypertension in mice (Satoh et al. 2006). Furthermore, we found that endogenous EPO and its receptor also play an important role in angiogenesis in response to hindlimb ischemia through upregulation of the system that involves vascular endothelial growth factor and its receptor, both directly by enhancing neovascularization and indirectly by recruiting endothelial progenitor cells and bone marrow-derived proangiogenic cells (Nakano et al. 2007).

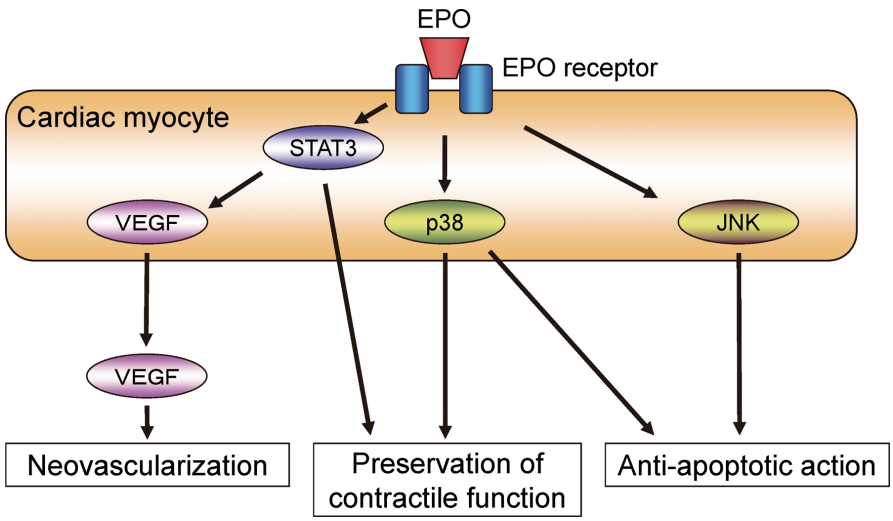

Fig. 1. Possible molecular mechanisms of protective effects of endogenous EPO/EPO receptor system on cardiac myocytes. Molecular mechanisms of protective effects of endogenous EPO/EPO receptor system on cardiac myocytes are proposed, based on the results of the experiments with transgene-rescued EPO-receptor null mutant mice developed by Suzuki et al. (2002). The endogenous EPO/EPO receptor system mediated activation of STAT3 and p38, VEGF production, and capillary growth have been shown in the murine model of left ventricular pressure overload (Asaumi et al. 2007). Anti-apoptotic action associated with the activation of p38 and JNK through the endogenous EPO/ EPO receptor system has been demonstrated in mice with myocardial ischemia followed by reperfusion (Tada et al. 2006). EPO, erythropoietin; STAT, signal transducers and activators of transcription; VEGF, vascular endothelial growth factor.

The results of these experiments using $EpoR^{-/-}{}_{\text{rescued}}$ mice, in which the EPO receptor is expressed exclusively in hematopoietic cells but not in nonhematopoietic cells including the cardiovascular system, clearly demonstrated that endogenous EPO-EPO receptor system in nonhematopoietic cells contributes to the protection against both myocardial and vascular injuries induced by various pathologic conditions.

Beneficial effects of endogenous EPO have also been reported in clinical studies. We prospectively studied 101 patients with first acute myocardial infarction who received successful primary percutaneous coronary intervention within 12 hours from the onset (Namiucni et al. 2005). Blood samples were collected to examine the serum EPO level following the primary percutaneous coronary intervention. We found that a high endogenous EPO level was a predictor of a smaller infarct size in these patients, which was independent of the hemoglobin content, creatinine level, oxygen saturation of the arterial blood, serum brain natriuretic peptide level, current smoking, presence of preinfarction angina within 24 hours from the onset of myocardial infarction, time to coronary intervention from the onset, etc. The results of our clinical study suggest that endogenous EPO plays a role in the protection against myocardial injury induced by ischemia and reperfusion in patients with acute myocardial infarction through a mechanism that is distinct from its hematopoietic action. The protective role of endogenous EPO against myocardial injury in patients with acute myocardial infarction has also been demonstrated by a clinical study by Niccoli et al. (2011). Niccoli et al. (2011) studied the association between circulating endogenous EPO and coronary no-reflow in patients with first ST-elevation myocardial infarction who underwent primary percutaneous coronary intervention and blood sampling to determine the serum EPO concentration, and found that decreasing EPO tertiles predicted angiographic coronary no-reflow. As no-reflow, which is observed after the release from epicardial coronary artery occlusion by successful percutaneous coronary intervention, has been shown to be associated with adverse clinical outcomes probably because of the increase in infarct size (Jaffe et al. 2010), endogenous EPO may exert a protective effect in patients with acute myocardial infarction who are subjected to primary percutaneous coronary intervention at least in part by the prevention of coronary no-reflow. Although the results of both clinical studies by us (Namiuchi et al. 2005) and by Niccoli et al. (2011) suggest that circulating endogenous EPO plays an important protective role against myocardial injury due to ischemia and reperfusion in patients with acute myocardial infarction, precise mechanisms of the protection remain to be clarified. It is still unknown whether circulating endogenous EPO in humans exerts a protective effect against the development of left ventricular systolic dysfunction due to chronic pressure overload and that of peripheral ischemia induced by peripheral artery diseases, in both of which endogenous EPO-EPO receptor system has been shown to play an important protective roles in animal experimental models (Asaumi et al. 2007; Nakano et al. 2007).

Protective effects of recombinant human EPO in animal models of cardiovascular diseases

The fact that EPO receptors are expressed not only in hematopoietic cells but also in nonhematopoietic cells suggests that recombinant human EPO, which is clinically used exclusively for erythropoiesis in patients with anemia, is potentially useful in the treatment of pathologic conditions of nonhematopoietic organs, such as the barin, heart, vessel, etc. Indeed, Sakanaka et al. (1998) reported that infusion of recombinant human EPO into the lateral ventricles of gerbils prevented ischemia-induced learning disability and rescued hippocampal neurons from lethal ischemic damage. Furthermore, Gorio et al. (2002) found that recombinant human EPO administered immediately after spinal cord injury elicited accelerated recovery of function, which was associated with the inhibition of inflammatory response and apoptosis.

In more recent studies, several investigators demonstrated that administration of recombinant human EPO elicits its cardioprotective effects in animal models of myocardial infarction. Calvillo et al. (2003) found that recombinant human EPO prevented apoptosis of cultured adult rat cardiomyocytes subjected to hypoxia and cardiomyocyte loss in a rat model of coronary artery ligation followed by reperfusion. Parsa et al. (2003) demonstrated that a single dose of recombinant human EPO reduced the infarct size and improved cardiac function in rabbits subjected to coronary artery ligation followed by reperfusion. The protection was associated with the activation of Akt and mitigation of cardiomyocyte apoptosis. Furthermore, Moon et al. (2003) found that a single dose of recombinant human EPO induced a reduction of cardiomyocyte apoptosis by 50% in rats subjected to coronary artery ligation without reperfusion. In their experiments, the reduction in apoptosis was accompanied by reductions in left ventricular size and functional decline. The results of these studies suggest that recombinant human EPO, when administered at the onset of acute myocardial ischemia, protects ischemic myocardium by amelioration of cardiomyocyte apoptosis in animal models of acute myocardial infarction. This is consistent to the result of our experiment (Tada et al. 2006) in which endogenous EPO-EPO receptor system in nonhematopoietic cells plays a protective role in myocardial ischemia and reperfusion at least in part by prevention of cardiomyocyte apoptosis. The protective effect of recombinant human EPO against acute ischemia followed by reperfusion was also confirmed in an isolated heart preparation model in which hearts were perfused with oxygenated buffer solution without blood (Cai et al. 2003; Cai and Semenza 2004; Wright et al. 2004), suggesting that blood cells are not essential for the protection by EPO.

Recombinant human EPO has also been shown to elicit a protective effect in infarcted hearts even when it was administered too late to protect myocardium against acute ischemic damage. Hirata et al. (2006) demonstrated that recombinant human EPO administered 6 hours after permanent coronary artery ligation in dogs improved left ventricular ejection fraction and reduced left ventricular end-diastolic pressure 4 weeks after the coronary artery ligation as compared with the treatment with saline. They also found that the improved left ventricular function was associated with increased capillary density in the ischemic area, suggesting that the protective effect by EPO can be explained at least in part by coronary angiogenesis. This is also consistent to the results our experiment using a different animal model with left ventricular pressure overload in which we demonstrated that endogenous EPO-EPO receptor system in nonhematopoietic cells elicited a protective effect against the exacerbation of left ventricular remodeling at least in part by the mechanism of coronary angiogenesis (Asaumi et al. 2007).

Protective effects of recombinant human EPO have also been reported in animal models of nonischemic heart diseases. Li et al. (2006) demonstrated that recombinant human EPO significantly attenuated doxorubicin-induced left ventricular dilatation and dysfunction in mice if EPO was started simultaneously with the initiation of doxorubicin. In this experiment, EPO protected myocardium against cardiomyocyte atrophy and degeneration, fibrosis, inflammation, and downregulation of several important sarcometric proteins, all of which were induced by doxorubicin. EPO did not show any cardioprotective effects when it was administered after the development of doxorubicin-induced cardiomyopathy. We recently reported that recombinant human EPO exerts cardioprotective effects against the development of cardiac remodeling and premature death induced by chronic left ventricular pressure overload due to transverse aortic constriction in mice (Wang et al. 2011). At 8 weeks after transverse aortic constriction, left ventricular diameter was increased, and left ventricular fractional shortening, an indicator of left ventricular systolic function, was decreased in mice treated by vehicle. Recombinant human EPO, which was initiated at 24 hours after the transverse aortic constriction and was continued twice a weak for 8 weeks, not only ameliorated the extent of left ventricular remodeling in terms of ventricular size and cardiac function but also improved survival. In contrast to the result of our previous study (Asaumi et al. 2007), in which protective roles of endogenous EPO-EPO receptor system was demonstrated using mice deficient in the EPO receptor exclusively in nonhematopoietic cells, the protective effects of recombinant human EPO cannot be explained by coronary angiogenesis.

Effects of recombinant human EPO and its derivative in patients with acute myocardial infarction

The results of the animal experiments described in the previous section strongly suggest that erythropoiesis stimulating agents (recombinant human EPO and its derivatives) administered before or immediately after

Table 1. Results of clinical trials that investigated whether erythropoiesis stimulating agents exerted cardioprotective effects in patients with acute myocardial infarction subjected to successful percutaneous coronary intervention.

Authors/ acronym and year	Patients	Dose and types of ESA	Effects on the primary end-point	Effects of the secondary end-point	Serious adverse events
Lipsic et al. 2006	22 patients with STEMI	300 μg of darbepoetin	No change in LVEF	N/A	None
HEBE III 2010	529 patients with STEMI	60,000 IU of epoetin alfa	No change in LVEF	No reduction in infarct size	Less adverse events
REVIVAL-3 2010	138 patients with STEMI	33,300 IU of epoetin beta × 3*	No change in LVEF	No reduction in infarct size	No significant increase
REVEAL 2011	222 patients with STEMI	60,000 IU of epoetin alfa	No reduction in infarct size	No change in LV remodeling	More adverse events

ESA, erythropoiesis stimulating agents; LV, left ventricular; LVEF, left ventricular ejection fraction; N/A, not applicable; STEMI, ST-segment elevation myocardial infarction. *Epoetin beta was administered immediately after percutaneous coronary intervention, and 24 and 48 hours later.

primary percutaneous coronary intervention in patients with acute myocardial infarction elicits some protective effects against myocardial ischemic injury. The first prospective randomized clinical trial to determine whether an erythropoiesis stimulating agent rescues myocardium in patients with acute myocardial infarction subjected to primary percutaneous coronary intervention was performed by Lipsic et al. (2006) using darbepoetin alpha, a long acting EPO analog. This was a feasibility and safety study, and only 22 patients were enrolled in the study. After a bolus injection of darbepoetin alpha before primary percutaneous coronary intervention, small and nonsignificant changes in hematocrit levels were observed, and no adverse events were recorded during the 30-day follow-up. Left ventricular systolic function determined by radionuclide ventriculography at 4 months after the injection of darbepoetin alpha revealed no significant improvement by the treatment as compared with no treatment (Table 1).

Several prospective randomized clinical studies with a relatively large number of patients (Table 1) followed the study by Lipsic et al. (2006). In HEBE III, a total of 529 patients with first acute myocardial infarction were randomized to receive either standard medical care alone, or in combination with a single bolus injection with recombinant human EPO (60,000 IU) within 3 hours after successful primary percutaneous coronary intervention (Voors et al. 2010). At 6 months after the administration of EPO, left ventricular ejection fraction did not show significant improvement as compared with standard treatment alone. Major adverse cardiac events including in-stent coronary thrombosis and re-infarction occurred more frequently in the control group as compared with the EPO group. Another prospective randomized trial, the Regenerate Vital Myocardium by Vigorous Activation of Bone Marrow Stem Cells (REVIVAL-3) study enrolled a total of 138 patients with first acute myocardial infarction subjected to primary percutaneous coronary intervention (Ott et al. 2010). Recombinant human EPO (33,300 IU) was administered immediately and at 24 and 48 hours after percutaneous coronary intervention. At 6 months after the treatment, left ventricular ejection fraction and infarct size both determined by cardiac MRI were not ameliorated by the treatment with EPO. More recently, the Reduction of Infarct Expansion and Ventricular Remodeling With Erythropoietin After Large Myocardial Infarction (REVEAL) trial, another prospective randomized trial enrolled 222 patients with acute myocardial infarction subjected to successful primary or rescue percutaneous coronary intervention (Najjar et al. 2011) who received a bolus injection of recombinant human EPO (60,000 I.U.) or placebo. Left ventricular infarct size determined by cardiac MRI within the first week of the treatment and 12 weeks after were similar between patients treated with placebo and those with EPO. There was a significant increase in the composite outcome of death, myocardial infarction, stroke, or coronary stent thrombosis in the patients who had received EPO as compared with those had received placebo. In a prespecified analysis of 21 patients aged 70 years or older, however, the infarct size determined within the first week of the treatment was significantly larger in the EPO group than in the placebo group.

Although recombinant human EPO exerts a protective effect against myocardial injury induced by ischemia and reperfusion in experimental animal models, EPO and its analog failed to show a beneficial effect in patients with acute myocardial infarction subjected to primary percutaneous coronary intervention enrolled in these clinical studies. The results of REVEAL suggest that recombinant human EPO may be even harmful in a certain clinical setting (Najjar et al. 2011). The reasons for the discrepancy in the results between animal experiments and clinical trials still remain to be clarified. One possible explanation for this may be very high doses of EPO used in the clinical trials for the patients with acute myocardial infarction as compared with those for patients with anemia in the real clinical situation. In this regard, Taniguchi et al. (2010) demonstrated that a relatively low dose of recombinant human EPO (6,000 I.U.) improved cardiac function and reduced

infarct size without any adverse events in patients with acute myocardial infarction subjected to primary percutaneous coronary intervention. Although this was a very small clinical study in which only 35 patients were enrolled, the results suggest that a relatively low dose of recombinant human EPO exerts a protective effect against myocardial injury induced by ischemia and reperfusion in patients with acute myocardial infarction. However, large-scale clinical studies are required to confirm the beneficial effects of a relatively low dose of recombinant human EPO in such patients.

Erythropoietin receptor as a novel therapeutic target in cardiovascular diseases

So much experimental evidence supporting the notion that both endogenous EPO/EPO receptor system and the administration of erythropoiesis stimulating agents exert protective effects in various animal models of cardiovascular diseases has been accumulated. However, most clinical trials that enrolled patients with acute myocardial infarction failed to demonstrate distinct favorable effects of erythropoiesis stimulating agents. In these clinical trials, very high doses of recombinant human EPO were administered to patients with acute myocardial infarction. In this regard, as mentioned in the previous section, attention should be paid to the clinical study by Taniguchi et al. (2010), in which 6,000 I.U. of recombinant human EPO was employed. In clinical studies that enrolled patients with chronic kidney disease and anemia, Drüeke et al. (2006) and Singh et al. (2006) demonstrated that normalizing hemoglobin levels using recombinant human EPO was associated with increased risks of cardiovascular events, the progression of chronic kidney disease, and poor quality of life as compared with targeting a lower hemoglobin level using a lower dose of EPO. Although it is not clear which is responsible for the unfavorable effects brought by normalizing hemoglobin levels, the relatively high hemoglobin levels achieved or the relatively high doses of EPO themselves, clinical studies that employ a relatively low dose of recombinant human EPO may settle the conflicting results between animal experiments and clinical studies.

Derivatives of EPO that exert neuroprotective effects but no erythropoietic action have been developed (Erbayraktar et al. 2003; Leist et al. 2004). Asialoerythropoietin, a nonerythropoietic derivative of EPO, is too short-lived to accelerate erythropoiesis that requires the continuous stimulation by EPO (Erbayraktar et al. 2003). The recent study by Ogino et al. (2010) showed that asialoerythropoietin mitigated left ventricular dilatation and dysfunction without affecting the hemoglobin levels in mice with chronic renal dysfunction and anemia. More recent study by Takeyama et al. (2012) demonstrated that asialoerythropoietin elicited cardioprotective effects in mice with doxorubicin-induced cardiomyopathy, those with large myocardial infarction, and those suffering from genetic cardiomyopathy. Such a compound that potentially activates the EPO receptor only in nonhematopoietic cells may exert favorable effects in patients with various cardiovascular diseases, which still remains to be elucidated in future clinical trials.

Conclusion

Both endogenous EPO-EPO receptor system and the administration of erythropoiesis stimulating agents, such as recombinant human EPO and EPO derivatives, have been shown to exert multiple protective actions in animal models of various cardiovascular diseases. However, most clinical trials failed to demonstrate favorable effects of high doses of recombinant human EPO on myocardial injury induced by ischemia and reperfusion in patients with acute myocardial infarction. Further clinical studies using low doses of EPO or derivatives of EPO without erythropoietic action are required to solve this important issue.

Conflict of Interest

The authors have no conflict of interest regarding this review article.

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