Editorial

Beyond erythropoiesis: The anti-inflammatory effects of erythropoietin

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See article by Li et al. [4] (pages 684–694) in this issue.

1. Cardioprotective effects of EPO

Erythropoietin (EPO) is a 30.4-kDa glycoprotein essential for red blood cell production. In adults, EPO is produced primarily in the kidney via an oxygen-sensing mechanism and stimulates erythroid progenitors in the bone marrow to increase red blood cell mass. In addition to its role in erythropoiesis, EPO has been shown to protect against ischemic injury in several organs including the heart. In various animal models, administration of EPO reduces infarct size and improves cardiac function following myocardial ischemia and reperfusion (I/R). The protective effects have been observed regardless of whether EPO was administered before ischemia, at the onset of ischemia, or at reperfusion [1,2].

The protective effects of EPO are also seen in chronic models of myocardial infarction (MI). In this regard, treatment of EPO before or immediately after coronary artery ligation decreases cardiac remodeling and improves myocardial function. Interestingly, a single dose of EPO (3000 IU/kg, i.p.) immediately after induction of MI improved cardiac function measured 8 weeks later. The therapeutic relevance of EPO was further demonstrated by van der Meer et al., who showed that EPO treatment started 3 weeks after MI improved cardiac function in a rat model of heart failure [3]. Consistent with this finding, in a report in this issue of *Cardiovascular Research*, EPO was started 6 weeks after induction of MI when heart failure was fully established in mice. Four weeks of EPO treatment diminished left ventricular (LV) dilatation and improved cardiac function [4].

EPO has anti-apoptotic effects in a variety of cell types including neurons, endothelial cells, smooth muscle cells, and cardiomyocytes [5]. EPO exerts a significant anti-apoptotic effect in cardiac tissue after ischemic injury. EPO also mobilizes endothelial progenitor cells (EPCs) from the bone marrow and acts as an angiogenic factor [6]. Both anti-apoptotic and angiogenic effects contribute to the cardiac protection of EPO during MI.

2. Anti-inflammatory effects of EPO

The inflammatory response following myocardial I/R includes neutrophil infiltration, increased oxidative stress, and production of inflammatory cytokines, which contribute to infarct size and cardiac dysfunction. Increased oxidative stress and cytokine expression have also been implicated in the development of heart failure post-MI [7].

EPO has anti-inflammatory effects. Recent studies by Rui et al. showed that pretreatment with EPO prevents the acute myocardial inflammatory response induced by ischemia and reperfusion [8]. EPO increases endothelial nitric oxide synthase (eNOS) protein expression and NO production in cardiomyocytes, which leads to decreased oxidative stress, neutrophil transendothelial migration, and myeloperoxidase activity in both in vitro and in vivo models of myocardial I/R. Consistent with this notion, Liu et al. showed that EPO not only decreases pro-inflammatory cytokine production including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and intercellular adhesion molecule-1 (ICAM-1), but also increases the expression of the protective cytokine IL-10 following myocardial I/R in rats [9].

In this issue of *Cardiovascular Research*, Li et al. extended the anti-inflammatory effects of EPO to a chronic heart failure model [4]. Mice were subjected to coronary artery ligation to induce MI. Six weeks after MI, EPO

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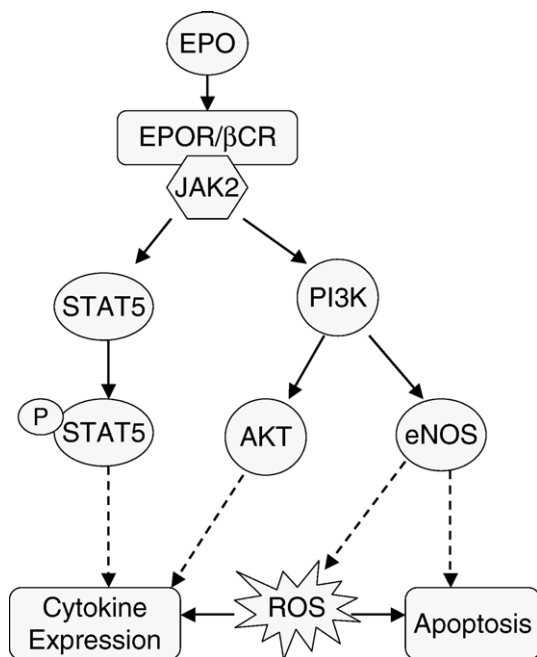 *E-mail address:* qfeng@uwo.ca.

Fig. 1. Intracellular signaling pathways of EPO leading to cardiac protection. EPO binds to a tissue-protective receptor consisting of the EPO receptor and the β common receptor (EPOR/βCR), and activates Janus tyrosine kinase 2 (JAK2). Activation of JAK2 phosphorylates signal transduction and activator of transcription 5 (STAT5), which inhibits cytokine expression in cardiac fibroblasts. In cardiac myocytes, however, JAK2 activates the phosphatidylinositol 3-kinase (PI3K) pathway, which inhibits cytokine expression via activation of Akt. PI3K also increases endothelial nitric oxide synthase (eNOS) expression and activity, which decrease reactive oxygen species (ROS) and cardiomyocyte apoptosis. Decrease in ROS may also attenuate cytokine expression and apoptosis. Dashed arrows indicate inhibitory effects.

treatment (1500 U/kg, s.c., twice a week) was started and continued for 4 weeks. Levels of inflammatory cytokines including TNF-α, IL-1β, IL-6, and transforming growth factor-β1 were reduced to near control levels. Inflammatory cell infiltration to the infarcted myocardium, oxidative damage in surviving cardiomyocytes, and fibrosis in the non-infarcted myocardium were all significantly attenuated. Importantly, the anti-inflammatory effects of EPO were accompanied by significant improvement in cardiac remodeling and function. Interestingly, these beneficial effects of EPO lasted for 4 weeks even after EPO treatment was stopped. The data suggest that EPO treatment attenuates or reverses the development of heart failure post-MI.

However, several important questions remain unanswered. First, does EPO treatment improve survival of mice with heart failure post-MI? The study by Li et al. had 10 mice in each group and no mortality was observed in any of the group from 6–10 weeks post-MI, which prevented them from studying the effects of EPO on survival. To address this question, a larger sample size and a longer follow-up are required. Second, does EPO treatment affect plasma or myocardial levels of endothelin-1 (ET-1) in these heart failure mice? EPO has been shown to induce ET-1

expression [10], which may hamper the beneficial effects of EPO as ET-1 is known to increase vascular resistance and induce cardiac hypertrophy. Finally, why was cardiomyocyte size not decreased after EPO treatment when all other parameters of cardiac remodeling including LV chamber size, filling pressure and ejection fraction were improved? Is this due to a technical limitation of myocyte size measurement using formalin-fixed tissue sections? Determination of myocyte size using freshly isolated ventricular cardiomyocytes, although technically demanding, may help to resolve this issue.

3. Signaling mechanisms of EPO in the heart

It is well known that erythropoietic effects of EPO are mediated by EPO receptors. However, the protective effects of EPO are mediated through the tissue-protective EPO receptor [11], a heterotrimer which consists of an EPO receptor and a β common receptor homodimer (EPOR/βCR). Cardiomyocytes express EPO receptors. Li et al. demonstrated that EPO treatment upregulated EPO receptors and increased phosphorylation of STAT3, STAT5, and Akt but not ERK in the failing myocardium [4]. In vitro experiments showed that the effects of EPO on H_2O_2-induced cytokine expression was inhibited by parthenolide, a specific inhibitor of STAT signaling in cardiac fibroblasts, while wortmannin, a selective inhibitor of PI3K/Akt signaling diminished protective effects of EPO against oxidative damage in cardiomyocytes. In addition, our recent studies demonstrated that EPO decreases oxidative stress and cardiomyocyte apoptosis via upregulation of eNOS, which is mediated by PI3K/Akt signaling [8,12]. These studies provide insights in the understanding of signaling mechanisms of EPO in cardiac myocytes. A summary of intracellular signaling pathways of EPO leading to cardiac protection is illustrated in Fig. 1.

4. Clinical significance

The anti-inflammatory and cardioprotective effects of EPO demonstrated in animal models suggest therapeutic potential of EPO in patients with acute MI and chronic heart failure. The biggest challenge lies in the translation of findings from animal models to clinical practice. Single-center clinical studies in patients with both acute MI and congestive heart failure have shown very promising results [13,14]. However, multi-center, large-scale clinical trials are required to determine the efficacy and safety of EPO in these patients as we continue to understand the fundamental biology of EPO in animal models.

Acknowledgement

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