# Erythropoietin in cardiac ischemia

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# Chapter 9

## Summary and conclusions

# Summary

Therapeutic strategies in the treatment of myocardial infarction (MI) are currently directed to shortening the time of ischemia by restoration of coronary blood flow ("open artery theory"). Although early reperfusion therapy of MI limits the extent of myocardial necrosis and improves the clinical outcome, morbidity and mortality after MI remains significant. Additional protection of myocardial cells during ischemia and reperfusion is needed to further limit cardiomyocyte death, with positive effect on cardiac function and prognosis of patients with acute MI ([1]).

Furthermore, most patients will at some time after MI develop heart failure, or left ventricular (LV) systolic dysfunction ([2]). As the five-years mortality of patients with a diagnosis of heart failure remains above 50% ([3]), strategies aimed at regeneration of failing myocardium may be required.

Traditionally viewed as a hormone stimulating hematopoiesis, erythropoietin (EPO) is recently considered to influence a broad array of cellular processes, that include protection against ischemic injury, angiogenesis and stem cell development ([4]).

In recent years, expression of functional EPO-R has been demonstrated in non-hematopoietic cells and organs including brain, kidney and cardiovascular tissue. These findings suggest that besides stimulating hematopoiesis, EPO may play a role as a pleiotropic survival and growth factor ([5]).

*Acute cardioprotection*

Many experimental studies have shown the protective effect of EPO in the brain. Systemic administration of EPO before or up to 6 hours after focal brain ischemia reduced the infarct volume by 50-75% ([6]). In **chapter 2** we examined the presence and functionality of EPO receptor in cardiac tissue and evaluated the effect of exogenous EPO administration on cardiac performance during ischemia/reperfusion (I/R). In the first part of the experiment we demonstrated the expression of EPO receptor in the rat heart, which is mainly present on endothelial cells and fibroblasts, and to a lesser extent on cardiomyocytes. Furthermore, the perfusion of isolated rat hearts (Langendorff set-up) with high concentration of EPO led to activation of important signaling pathway- MAPK, which was implicated in cell survival after reperfusion injury in the heart ([7]). In the second part of the study, administration of exogenous EPO during I/R resulted in improved recovery of left ventricular pressure and coronary flow. Moreover, this was associated with a 56% reduction of cellular damage during reperfusion and diminished apoptosis.

In the last few years, different groups demonstrated that administration of EPO during *in vivo* cardiac I/R and permanent coronary ligation led to infarct size reduction associated with mitigation of apoptosis ([8;9]). Early reduction in infarct size and apoptosis inhibition was also associated with subsequent prevention of LV dysfunction ([10]). However, the clinically relevant question of therapeutic window for EPO-mediated cardioprotection during I/R in the heart remained to be answered. We addressed this issue in **chapter 3**, administrating EPO 2 hours before I/R, at the start of ischemia and after the onset of reperfusion. A single dose of EPO at different time points resulted in 19-23% reduction in infarct size, which was accompanied by an improved LV function. Furthermore, EPO reduced the number of cells entering apoptosis but had no effect on proliferative activity in myocardium. Thus rather than stimulating cell proliferation, EPO inhibits the programmed cell death associated

with acute I/R injury. Importantly, this effect could not be ascribed to hematocrit increase, since this would occur not before 2-3 days after the EPO administration. The extension of cardioprotective effects of EPO beyond the start of reperfusion provides a broad "window of opportunity" for potential treatment of acute coronary syndromes.

### *Post-MI Neovascularization and Regeneration*

Besides its acute cardioprotective effects, through inhibition of apoptosis, EPO may also influence formation of new vessels (neovascularization) after ischemic injury. In a rat model of stroke, EPO treatment initiated 24 hours after the occlusion of cerebral artery improved neurological function and increased the density of cerebral microvessels in the stroke boundary ([11]). In the heart, neovascularization in the non-infarcted hypertrophied part of the myocardium, may improve cardiac function and attenuate post-MI LV remodeling ([12]). In **chapter 4** we assessed the impact of EPO treatment on new vessels formation in a post-MI heart failure. We confirmed previous findings, that administration of EPO immediately after the induction of MI reduces the infarct size, which was associated with improved hemodynamics. More interestingly, EPO treatment started three weeks after MI, despite not affecting the infarct size, resulted in improved cardiac performance, as shown by a 17% increase in LV developed pressure and 46% decrease in N-ANP levels. This improvement was associated with restoration of capillary density to sham levels and significant increase in capillary-to-myocyte ratio, indicating neovascularization. Furthermore, these beneficial effects were also related to increased percentage of alpha-MHC (myosine-heavy chain) isoforms, a molecular marker of enhanced myocardial contractility.

Although our study showed apparent effect of EPO on formation of new blood vessels after MI, several questions remained unanswered. Hematocrit increased by 27% in the EPO treated rats, and although less likely, the elevated oxygen-carrying capacity of blood may have contributed to improved cardiac performance. In addition, when applied to clinical situation, chronic high-dose EPO treatment may cause hypertension, seizures, vascular thrombosis and death ([13]). This would be even of greater concern in patients with already elevated cardiovascular risk. Moreover, the mechanism behind the effect of EPO on neovascularization remains largely unknown. Both stimulation of *in situ* endothelial cells proliferation or mobilization of endothelial progenitor cells (EPCs) derived from the bone marrow may play a role. Recently, increased levels of circulating EPCs were associated with reduced risk of death from cardiovascular causes in patients with confirmed coronary artery disease ([14]). Importantly, EPO was shown to stimulate the mobilization and functional activity of EPCs ([15]).

To discern the hematopoietic from non-hematopoietic effects of EPO and study the possible mechanisms involved, we compared low- vs. high-dose EPO treatment in a post-MI heart failure rat model (**chapter 5**). We assessed cardiac function over time, EPCs mobilization and neovascularization. Hematocrit increased with high- but not with low-dose treatment. In both EPO-treated groups, serial echocardiography demonstrated preservation of left ventricular systolic function during 9-weeks long follow-up, and hemodynamic measurements revealed improved cardiac contractility and relaxation at the end of the study. In addition, in EPO-treated groups the number of circulating EPCs was significantly increased, which was associated with a 42% increase in capillary-to-myocyte ratio in high-dose and 28% in low-dose group. This is in line with a study performed in a rat model of progressive renal disease, where treatment with a low-dose EPO conferred tissue protection and preserved capillary network in the kidney, but did not raise hematocrit ([16]).

Thus in situations where prolonged treatment is warranted (heart failure), low-dose EPO may maintain to be beneficial, and at the same time avoid the occurrence of adverse effect associated with hematocrit increase.

### *Clinical Implications*

EPO has been used in clinical medicine for more than two decades, initially to treat anemia caused by reduced EPO production related to chronic kidney disease. The indications for EPO treatment have extended in the last years to include also anemia in patients with myelodysplastic syndromes, cancer patients treated with chemotherapy, and prophylactic treatment to reduce the need for transfusions before major surgery ([17]). In cardiology, EPO treatment in chronic heart failure patients with anemia has been shown to improve LV ejection fraction ([18]) and enhance exercise capacity ([19]). However, although the range of indications for EPO treatment is becoming broader, so far the main objective of the therapy is to increase hematocrit in anemic patients. Nevertheless, non-hematopoietic effects of EPO may also be beneficial in non-anemic patients experiencing acute ischemic event. Indeed, a first randomized, clinical trial in stroke patients showed that beyond being safe and well tolerated, EPO treatment also improves clinical outcome and ameliorates the ischemic brain damage ([20]).

In heart, while there is a rapidly increasing number of studies which suggest a protective role of EPO treatment in experimental ischemia, so far no clinical data are available. Interestingly, higher levels of endogenous EPO were shown to be independently associated with smaller infarct size in patients with acute MI ([21]). In **chapter 6** we performed a pilot clinical study with EPO treatment in acute MI patients. The primary objective of this single center, investigator-initiated, randomized study was to evaluate the safety and tolerability of long-acting EPO analogue darbepoetin alfa treatment in non-anemic patients with acute ST-elevation MI treated with primary coronary angioplasty (PCI). Twenty-two patients were randomized to receive one bolus of 300 μg long-acting EPO analogue darbepoetin alfa or no additional medication before PCI. Administration of darbepoetin alfa in patients was both safe and well tolerated. We did not encounter any adverse events associated with darbepoetin treatment. Rather than increasing the hematocrit above reference values, one bolus of darbepoetin merely prevented the decrease in hematocrit observed in the control group. Importantly, darbepoetin treatment led to a significant elevation of EPCs, 72-hours after the drug administration. Despite non-significantly longer "time to treatment" and more extensive baseline area at risk (cumulative ST-elevation) in the darbepoetin-treated group, LV function 4 months after the MI was similar in both groups. The results of this first pilot study support a larger scale clinical trial to establish efficacy of EPO administration in patients with acute MI.

Already for a long time, anemia is recognized as a risk factor for cardiovascular (CV) outcomes in patients with chronic kidney disease and heart failure. Recently, anemia was associated also with reduced survival in patients with established and stable coronary artery disease ([22]). In **chapter 7**, we examined the effect of hemoglobin levels on short-term CV mortality in patients with acute MI. In a retrospective study, we analyzed 1841 consecutive patients admitted with the diagnosis of acute MI. We found a statistically significant increase in 30-day mortality in patients presenting with hemoglobin lower than 10 g/dl. Although other risk factors (age, renal function) were more strongly associated with the survival, and could in part explain the effect of anemia, low hemoglobin levels remained independent predictor of 30-day mortality even after adjustment for these variables. Specific therapeutic strategies, including EPO treatment, in such anemic MI patients should also be further considered.

## Conclusions

A traditionally hematopoietic hormone erythropoietin is increasingly recognized as a pleiotropic cytokine, with effects reaching further that stimulating red blood cells production (discussed in **chapter 8**).

The detection of EPO receptor expression outside the hematopoietic system implicated the possibility of various non-hematopoietic functions of EPO. In brain, EPO was shown to confer neuroprotection against stroke and other neurological disorders. Beneficial effects of EPO during ischemic injury were documented also in various other organs and tissues.

Recombinant form of EPO has been used in clinical setting for almost two decades to treat different forms of anemia. Although treatment with EPO is generally well tolerated and safe, it may be associated with adverse effects, mainly related to hematocrit increase ([13]). The recently synthetized non-hematopoietic variants of EPO, which retain tissue protective activity, may prove to be both effective and safe alternative for non-anemic patients with organ ischemia ([23]).

In heart, EPO appears to influence two crucial processes during experimental cardiac ischemia/reperfusion injury, first by acutely inhibiting the apoptosis and reducing the infarct size, and second by promoting neovascularization and preserving the myocardial structure and function over longer time frame. If these effects could be extrapolated to heart patients, EPO treatment of acute coronary syndromes may be able to "kill two birds with one stone".

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