Erythropoietin in cardiac ischemia

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Chapter 1

Introduction and aims of the thesis

General introduction and aims of the thesis

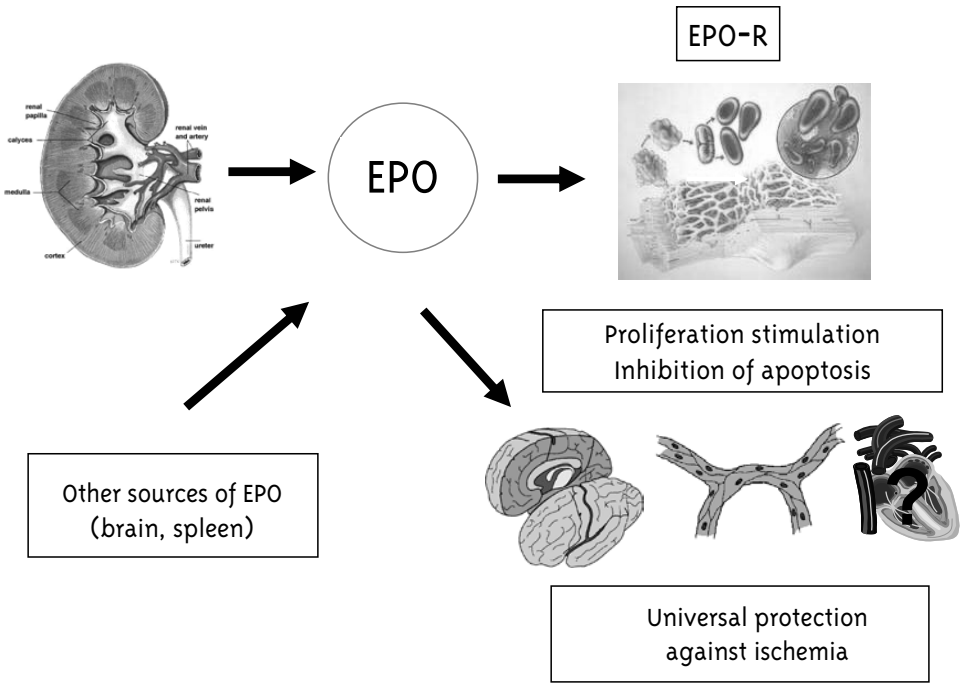
Introduction

Myocardial infarction (MI) is the major cause of death in the Western world. Currently, the emphasis in the treatment of MI is on early reperfusion. Although timely reperfusion is a prerequisite for salvaging of myocardium, the restoration of blood flow to previously ischemic cells results in number of deleterious effects referred to as reperfusion injury ([1]). Additional interventions that preserve the viability during ischemia and reperfusion may further limit the extend of acute MI and improve the clinical outcome ([2]).
Still, myocardial infarction and consequent loss of contractile myocardium is a frequent cause of chronic heart failure (CHF). Approximately half of patients with acute MI would develop heart failure, either during hospital admission or over longer time period ([3;4]). In the Framingham population, MI accounts for 34% cases of CHF in men and 13% in women ([5]). Paradoxically, the declining mortality after an acute MI together with improved post-MI therapy, may contribute to the increasing prevalence of CHF ([6]). Current pharmacological treatment of CHF is focused on suppressing the neurohormonal systems, chronic stimulation of which leads to deterioration of heart function. However, despite this "optimal" therapy, the patients diagnosed with heart failure have a poor prognosis ([7]). Half of the patients die within 4 years, and in patients with severe heart failure over 50% will die within 1 year ([8]). Importantly, conventional medical strategies for post-MI heart failure do not attempt to correct the underlying cause (i.e. damaged myocardium), raising a need for strategies aimed at myocardial regeneration and repair ([9]).

Erythropoietin

Erythropoietin (EPO) is a glycoprotein hormone produced primarily in the kidneys, traditionally known to stimulate hematopoiesis. Synthesis of EPO is upregulated by hypoxia and mediated by hypoxia-inducible factor-1 (HIF-1) ([10]). In bone marrow, EPO acts on a specific receptor (EPO-R), with subsequent activation of various signaling pathways (STAT5, MAPK, PI3/Akt)([11]). Interestingly, rather than stimulating proliferation, activation of these pathways leads to inhibition of programmed cell death (apoptosis) ([12]). EPO thus acts primarily as a survival factors for erythroid progenitor cells, and in this manner increases the number of mature red blood cells in the circulation. Cloning of human EPO and production of recombinant human EPO (rhEPO) represented a breakthrough in the treatment of anemia caused by EPO deficiency due to chronic kidney disease ([13]). At present, rhEPO is approved also for treatment of anemia caused by other conditions, including anemia associated with chemotherapy, HIV antiviral treatment or to reduce the need for transfusion in perioperative surgical patients ([14]). Recently, a novel erythropoiesis stimulating factor (NESP, darbepoetin) has been synthesized ([15]), containing higher content of carbohydrates than rhEPO, rendering this molecule a longer plasma half-life with obvious advantages regarding administration frequency.

Figure: Pleiotropic effects of erythropoietin (EPO); EPO-R, erythropoietin receptor.



Pleiotropic functions of erythropoietin

Hematopoiesis was for long considered to be the only function of EPO. Recent detection of EPO and EPO-R expression outside the hematopoietic system (endothelial cells, neurons, trophoblast cells) suggested also other physiological functions of EPO ([16]). Similar to bone marrow, EPO may act as a survival factor also in other organs, preventing cell death and promoting tissue regeneration (figure).

In particular, the presence of EPO and EPO-R in central nervous system prompted further research into the non-hematopoietic roles of EPO. Both *in vitro* hypoxia and *in vivo* ischemia stimulates EPO and EPO-R expression in neuronal cells ([17;18]). In the adult human brain, the EPO-system is present in a state of "dormancy", with pronounced upregulation of EPO and EPO-R expression after ischemic injury ([19]), suggesting an endogenous neuroprotective function of EPO. These findings imply also a possible therapeutic potential for EPO in a setting of cerebral ischemia.

In a rat stroke model, administration of EPO was shown to significantly decrease the volume of damaged brain, even when administered up to 6 hours after cerebral artery occlusion ([20]). The reduced ischemic brain damage corresponded to salvaged tissue around the ischemic core (irreversible damage), which is related to reperfusion injury. Inhibition of neuronal apoptosis has been demonstrated as a mechanism explaining these neuroprotective effects ([21]). Also in other organs (kidney, retina) EPO was shown to reduce the extent of ischemia/reperfusion injury, largely by mitigating apoptosis ([22;23]).

Protection against ischemia and reperfusion injury in the heart

Infarct size is a major determinant of prognosis in patients after acute MI ([24]). Early reperfusion with percutaneous coronary stenting or thrombolytic therapy remains so far the best strategy to reduce infarct size ([2]) and is associated with improved short and long-term survival ([25]). However, reperfusion may be viewed as a "double edged sword", as it may initiate additional myocardial injury beyond that generated by ischemia alone ([26]). This is referred to as "reperfusion injury" and is manifested by myocardial stunning, endothelial dysfunction and irreversible cellular damage ([27]). Animal models of sustained ischemia have shown exacerbation of myocardial injury during reperfusion, mediated largely by cytotoxic effects of free radical generation, complement activation, shifts in substrate use and inflammation ([28]). These changes occur both within the already irreversibly damaged myocardium, but reperfusion may also lead to conversion from reversible to irreversible injury in a population of severely impaired myocytes ([29]).

Two forms of cell death are implicated during ischemia and reperfusion in the heart, namely necrosis and apoptosis ([1]). Although the exact contribution of these two forms of death is still under discussion, apoptosis progressively develops and accelerates during the reperfusion ([30;31]). Furthermore, apoptosis has been detected in the heart not only during acute MI, but may also contribute to progressive loss of surviving cells during subacute and chronic ischemic stages ([32]). Targeting anti-apoptotic mechanisms of cellular protection at the time of reperfusion may therefore offer a potential approach to attenuate reperfusion-induced cell death ([33]). In conclusion, although early reperfusion with primary coronary intervention and stenting in the management of acute MI salvages greater amount of myocardium than is irreversibly damaged by reperfusion injury, additional cell protection may provide even greater benefits in terms of infarct size reduction and improvements in clinical outcome.

Repair of the failing heart

Recently, a decades old dogma declaring an inability of myocardial regeneration has been revised. Experimental studies and early-phase clinical trials have made the futuristic dream of heart repair an achievable therapeutic goal ([9]). Several concepts for cardiac repair have been proposed, among which implantation of cells capable to replace cardiomyocytes and/or myocardial vasculature ([9]). The most promising results have been obtained with recruitment of bone marrow-derived stem cells into the area of infarction. Although transdifferentiation of these cells into cardiomyocytes has been suggested ([34]), it appears very limited in an *in vivo* situation and other mechanisms seem more plausible. Stem cells may release paracrine mediators that inhibit apoptosis or enhance endogenous repair mechanisms in the heart ([35]). Most likely, stem cells may stimulate neovascularization, leading to augmented oxygen tissue supply. Neovascularization may be mediated by physical incorporation of bone marrow-derived endothelial progenitor cells (EPCs) into new capillaries ([36]) or by angiogenic cytokines (VEGF) secreted from these cells that potentiate angiogenic activity of endogenous cells ([37]). EPCs stimulated neovascularization of the peri-infarct zone in the heart was shown to prevent ventricular remodeling and improve cardiac function ([38;39]).

Although the mechanism of stem (progenitor) cells therapy is far from being understood, numerous clinical studies with bone marrow-derived cells have already been performed.

Besides establishing safety and feasibility, preliminary efficacy data also suggest that stem cell therapy has the potential to improve myocardial perfusion and contractile function in patients after acute MI, coronary artery disease or heart failure ([35]). In the BOOST trial, intracoronary infusion of autologous bone marrow cells after myocardial infarction improved the global left ventricular ejection fraction 6 months after cell transfer ([40]). However, other trials have provided conflicting results with many open questions, and cautiousness in rapid translation of experimental results to clinical situation is warranted. If there exists also an opposite direction from bench to bedside, it should be probably (at least partly) applied in this field. Erythropoietin was also shown to mobilize EPCs from bone marrow and enhance ischemia-induced neovascularization ([41]). In addition, EPO may also promote new capillary formation from preexisting vessels into ischemic area ([42]). Capillary growth has been observed in rat aortic rings after incubation with EPO ([43]) and also in endothelial cells derived from myocardial tissue ([44]).

Aims of the thesis

This thesis focuses on the non-hematopoietic effects of EPO in the heart. The two main aims of the experimental part of this thesis were:

1. To establish the presence and functionality of EPO system in the heart and to further study the effect of EPO administration on cardiac ischemia/reperfusion injury.
2. To investigate the influence of EPO treatment on neovascularization in a postischemic heart failure.

Therefore we employed experimental models of ischemia/reperfusion injury (**chapter 2,3**) and post-MI heart failure (**chapter 4,5**). Furthermore, we aimed to elucidate the mechanisms associated with both acute and chronic effects of EPO in the heart. Importantly, we seeked to separate the hematopoietic and non-hematopoietic effects of EPO treatment on heart structure and function.

In the clinical part of the thesis, we intended to translate the results of the experimental studies into first clinical, randomized study to assess the safety and feasibility of EPO administration in patients with acute MI **(chapter 6).**

Because anemia is associated with worse prognosis in heart failure and stable coronary artery disease, in **chapter 7** we studied the prognostic value of low hemoglobin levels on short-term mortality in patients with acute MI. **Chapter 8** summarizes the "state-of-art" of EPO-mediated cardioprotection and presents future perspectives on this clinically relevant topic.

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