Erythropoietin in the Treatment of Heart Failure and Ischemia

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Summary

Introduction

Erythropoietin (EPO) is a glycoprotein of the molecular weight of 30.4kD comprising 165 aminoacids. Its structure has been for the first time roughly characterised by Kuratowska et al [1964]. Its gene has been cloned and the structure finally described by Jacobs et al [1985] and Lai et al [1986]. EPO is expressed in and released mostly from the kidney [Fisher et al 1961; Jakobson et al, 1957; Kuratowska et al, 1961] by the peritubular fibroblasts of the cortex [Maxwell et al., 1993]. The main role of EPO is stimulation of erythropoiesis by stimulation of proliferation and maturation as well as inhibition of apoptosis of the erythroidal progenitor cells of the bone marrow. Recombined human EPO (rhEPO) is used in the treatment of anaemia in the patients suffering kidney failure and in some other anaemias. However, more recent works have shown that EPO is expressed also outside the kidney and that it has pleiotropic effects in tissues and cells other than those of the bone marrow. EPO is expressed (albeit in the amounts much less then in the kidney) in the neurons and astrocytes of the brain, in the liver and in the uterus where it plays a role of the local paracrine factor [Coussons et al., 2005].

In the fetal life the receptors for EPO (EPO-R) are expressed in all body tissues. After birth their expression is preserved in the erythroid progenitor cells of the bone marrow, but also in the neurons and astrocytes of the brain, in the vascular endothelial and smooth muscle cells as well as in myocytes and fibroblasts of the heart [Jie et al,2006; Schwartzenberg et al., 2006]. Recently the literature concerning EPO is rapidly expanding since the experimental and clinical studies have shown that rhEPO has cytoprotective effects in the brain strokes [Ehrenreich, 2002] and in the myocardial ischemia and failure.

Experimental studies

**Cellular sygnaling pathways activated by EPO binding to its receptors.**

The effects of EPO result from its binding to a specific receptors. The receptor (EPO-R) is not or is complexed with a homodimer, the common β receptor (βcR) known also as CD131. Erythropoietic effect of EPO results from its binding to EPO-R of the erythroid progenitor cells of the bone marrow. Recombined human EPO (rhEPO) is used in the treatment of anaemia in the patients suffering kidney failure and in some other anaemias. However, more recent works have shown that EPO is expressed also outside the kidney and that it has pleiotropic effects in tissues and cells other than those of the bone marrow. EPO is expressed (albeit in the amounts much less then in the kidney) in the neurons and astrocytes of the brain, in the liver and in the uterus where it plays a role of the local paracrine factor [Coussons et al., 2005].

EPOβcR activates a number of signaling pathways. The initial link of two of them is tyrosine kinase JAK2. JAK2 phosphorylates a transcription factor STAT5, which inhibits expression of proinflammatory cytokines [Bullard et al.,2005; Feng, 2006]. Jak2 activates also kinase P13K, which activates kinase Akt. Its final effect is inhibition of expression of proinflammatory cytokines. Moreover, Akt inhibits glycogen synthase kinase (GSK-8), the activation or overexpression of which enhances apoptosis [Nishihara et al., 2006]. P13K activates also eNOS thus limiting the oxydative stress [Feng, 2006]. P13K besides activating Akt, activates and initiates translocation from cytosol to sarcolemma of protein kinase C (PKCs) [Hanlon et al., 2005].

**Experimental cardiac infarction**

In most papers (except for one [Hale et al, 2005]) EPO was reported to exert a strong protective effect in the experimental myocardial infarction. A single injection of EPO immediately after ligation of the left coronary artery in the dogs resulted in three fold reduction of infarct area investigated four
weeks later [Hirata et al., 2006]. Ejection fraction of the left ventricle (EF) was significantly larger in the animals which received EPO injection. Similar results were obtained in rats. A single injection of EPO within one hour after ligation of the left coronary artery reduced the infarct area investigated 4-5 weeks later by 15%-50%, and prevented decrease in the EF and left ventricular dilatation [Moon et al., 2003; Moon et al., 2005; Moon et al., 2006a; Moon et al., 2006b; Nishiya et al., 2006; Hanlon et al., 2005; Fiordaliso et al., 2005]. Similar effects of EPO were reported also in mice with ligated coronary artery [Li et al., 2006; Prunier et al., 2005]. The myocardial protection of EPO most likely results from its effects on apoptosis and coronary perfusion. EPO has been shown to strongly inhibit apoptosis induced by staurosporin in cultured myocytes [Moon et al., 2006b; Fiordaliso et al., 2005] and to reduce by about 50% the number of apoptotic cells in area at risk in the in situ hearts [Moon et al., 2003; Moon et al., 2005; Nishiya et al., 2006].

EPO increased coronary perfusion in the area at risk and in the intact parts of the ventricle and increased the capillary density. Stimulation of angiogenesis most probably resulted from release by EPO from the bone marrow of the endothelial progenitor cells (EPC). Their markedly increased numbers have been observed in the peripheral blood [Hirata et al., 2006; Nishiya et al., 2006; Prunier et al., 2005; van der Meer and Lipsic, 2006]. EPC may stimulate angiogenesis either by docking in capillaries and differentiation into mature endothelial cells or by release of pro-angiogenic cytokines.

EPO may protect myocytes also by means of antiinflammatory effects. It has been found that in mice with ligated coronary artery EPO blocked expression of inflammatory cytokines interleukin (IL)-ß, IL-6, TNFα and TGF-ß and reduced damage of DNA by oxydatitive stress. Similar results were obtained in cultured neonatal myocytes exposed to H2O2 [Li et al., 2006]. It is probably due to antiinflammatory and antioxydative properties of EPO that it potentiates ischemic preconditioning [Nishihara et al., 2006].

The work of Tada et al. [2006] addressed the question whether EPO is a factor involved in the intrinsic physiological and pathological mechanism or its effects are rather pharmacological. These workers developed mice deprived of EPO-R in non erythroid lineage cell (i.e. in cardiac myocytes among others). In Tg mice subjected to 30 min ischemia and reperfusion deeper hemodynamic deterioration of the left ventricles, larger infarction area, greater hypertrophy, and larger count of apoptotic myocytes was found then in the control normal mice. These results clearly show that endogenous EPO exerts a permanent protective effect in the heart.

It was also interesting to check whether the beneficial effects of EPO are related to stimulation of erythropoiesis or to the direct action in the cardiac myocytes. An excellent tool for answering this question has been provided by synthesis of carbamylated EPO (CEPO), which does not bind to the EPO-R but binds to the EPO0cR. Hence CEPO does not affect the erythroid progenitor cells of the bone marrow but may exert its protective effects elsewhere. A single dose of rhEPO or CEPO was injected into rats immediately after ligation of the left coronary artery. Twenty four hrs later in both groups of animals the number of apoptotic myocytes in the area at risk was reduced by 50% with respect to infarcted hearts of the animals which did not receive rhEPO or CEPO. Four weeks after surgery the area of the scar was 4 fold less in both groups of the treated animals then in the control ones. In the treated groups the diastolic and systolic volumes of the left ventricles and EF did not change whereas in the not treated groups the volumes of the left ventricle largely increased and EF decreased by more then 50%. There were no differences between the groups receiving rhEPO or CEPO. Both CEPO and rhEPO equally inhibited apoptosis induced by staurosporin in the cultured myocytes. Hematocrite was increased by rhEPO by 5.1% whereas it was not affected by CEPO [Moon et al., 2006b]. In another work [Fiordaliso et al., 2005], CEPO injected before closing of the coronary artery in rats for 40 min and subsequently once a day for 6 days decreased the infarct area by 17% - 23%, inhibited myocyte hypertrophy, decresed the left ventricular diastolic pressure and enhanced the response of the heart to dobutamine. Also in this paper inhibition by EPO of apoptosis induced in the culture of adult myocytes by staurosporin was reported.

Thus the above papers provide convincing evidence showing that EPO exerts its protective effects by the direct action in the cardiac myocytes.

In some papers two time windows for the optimal effects of EPO were reported: early and delayed. It seems that the best early effects were obtained when EPO was injected just before or within one hr after ligation of coronary artery. Its effectiveness rapidly decreases and after 12 hrs weak effects could be obtained only by very high doses. After 24 hrs after surgery a single dose of EPO was ineffective [Hirata et al., 2006; Moon et al., 2003; Moon et al., 2006a]. In another work [Fiordaliso et al., 2005], CEPO injected before closing of the coronary artery in rats for 40 min and subsequently once a day for 6 days decreased the infarct area by 17% - 23%, inhibited myocyte hypertrophy, decresed the left ventricular diastolic pressure and enhanced the response of the heart to dobutamine. Also in this paper inhibition by EPO of apoptosis induced in the culture of adult myocytes by staurosporin was reported.

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Preventive effect of erythropoietin on cardiac dysfunction in doxorubicin-induced cardiomyopathy

Doxorubicin is a very effective antineoplastic drug, however, its clinical use is limited by its harmful
Clinical studies
Whereas preclinical studies concerned mostly the effects of EPO in myocardial infarction, the clinical studies addressed mainly the use of EPO for treatment of chronic heart failure (CHF).

According to various statistics 15% - 65% of patients with CHF suffer anemia defined as the level of Hb < 12 g/dL. Anemia was proved to be in these patients an independent risk factor for rehospitalization and mortality [Szachiewicz et al., 2003; Silverberg et al., 2005]. Decrease in Hb level positively correlates with deterioration of systolic and diastolic LV function, dyspnea and effort tolerance. In these patients CHF is complicated by progressing renal failure [Silverberg et al., 2005].

Mechanism of heart damage by schema
The tissues hypoperfusion results in vasodilatation and hypotonia which stimulate the sympathetic nervous system and ATII release. Therefore anemia may be an independent factor inducing cardiac hypertrophy the outcome of which is worsened by limited oxygen supply. The kidneys are also hypoperfused which leads to deterioration of their function up to the failure.

Mechanism of anemia in CHF
Hypoperfusion of kindeyes stimulates EPO expression, the level of which is in CHF increased in peripheral blood. However, the increase in hematopoiesis is inadequate to the degree of anemia and does not compensate it. There are several reasons for this inadequacy. Amount of released EPO is disproportional to the degree of anemia. This results most probably from increased expression of inflammatory cytokins in CHF. These proteins (mostly TNFα) inhibit EPO expression and desensitise erythroid progenitor cells to EPO. Paradoxically, some therapeutic measures may deepen inadequacy of EPO release with respect to the degree of anemia The expression and release of EPO by the peritubular fibroblasts in the renal cortex is stimulated by the sympathetic nervous fibres. Decrease in ATII synthesis by inhibitors of the converting enzyme or blocking of AT1 receptors by sartanes results in decrease of the level of activation of the sympathetic system thereby leading to decreased release of EPO. Another factor responsible for anemia in CHF is the disturbed iron turnover.

EPO in the treatment of congestive heart failure (CHF)
Three forms of erythropoiesis stimulating proteins are currently used for the patient treatment: epoetin-α, epoetin-β and darbepoetin-α. Epoetins are the recombinant human EPO. Their half time in blood plasma after a single injection is 6-8 hrs. Darbepoetin is an analog of the human EPO of the prolonged activity. Its affinity for EPO-R is higher and the half time in blood serum longer (48 hrs) then those of epoetins. The dosage of EPOs varied from 1500 – 3000 u/week in single or divided doses over several weeks to few months. Treatment with EPO was usually supported by iron supplementation.

The early investigations were performed in small groups of patients suffering CHF of NYHA class III-IV accompanied by anemia (Hb<12/dL). Parmanent increase in Hb level [Cleland et al.,2005; Mancini et al., 2005; Silverberg et al.,2005], increase of EF, shift in NYHA class [Silverberg et al., 2005], as well as increase in exercise tolerance expressed as VO2 and duration of exercise,and improvement of quality of life [Mancini et al.,2003] were reported. Decrease of the number of rehospitalizations and reduction of the use of diuretics was also reported [Silverberg et al., 2005]. However, the results of more recent randomized, double-blind, placebo controlled trials are less optimistic. In the study of Ponikowski et al [2007] patients receiving darbepoetin-α had an improvement in self-reported Patient's Global Assessment of change but no significant change in the Kansas City Cardiomyopathy and Minnesota Living with Heart Failure Questionnaire scores. Hb concentrations were increased and maintained. However, only a trend toward increased exercise time and no change in the peak VO2 was observed. There was no shift in NYHA class, but the number of rehospitalizations was decreased. Likewise in a subgroup analysis of the darbepoetin-α studies presented at the European Society of Cardiology Heart Failure meeting in June 2006, reported only non-significant improvements in symptom-related endpoints. There was no difference in the incidence of serious adverse events or deaths between the groups receiving darbepoetin or placebo. Darbepoetin was well tolerated [Coletta et al, 2006].

To my knowledge based on PUBMED only one paper adressing treatment of patients with myocardial infarction (MI) with EPO was published [Lipsic et al., 2006]. A single dose of 300 µg of darbepoetin in 22 patients admitted with the first acute MI and treated with primary PCI resulted within 72 hrs in large increase in the count of the endothelial progenitor cells of the bone marrow in the peripheral blood, and insignificant increase of hematocrite. Four months after infarction there was no difference in LVEF between the EPO and placebo groups.

Conclusion
The combined preclinical and clinical studies concerning use of EPO for the treatment of CHF seem encouraging, but further investigation on dosage, timing, and stage of development of CHF optimal for treatment is urgently needed.

Summary
Over the last decade it has been found, that erythropoietin (EPO), a glycoprotein expressed mainly in and released from the kidney, besides stimulating erythropoiesis has a pleiotropic, cytoprotective effects in brain and heart. In this paper we present the preclinical studies showing that EPO injected soon after experimental infarction dramatically reduces its area, improves LV hemodynamics and inhibits post-infarction remodeling. Administered several weeks after infarction, EPO improves LV hemodynamics and reduces hypertrophy. EPO was proved to inhibit apoptosis, to block expression of proinflammatory cytokines and to stimulate angiogenesis by mobilization of the endothelial progenitor cells from the bone marrow. Its beneficial effects consisting of increase in Hb level, increase in peak VO₂ and exercise tolerance as well as improvement of the quality of life in patients suffering chronic heart failure have been reported.

Bibliography

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CV of the author
- Graduated in 1952 from University Medical School in Warsaw, received PhD degree in 1964 and docent degree in 1966.
- In 1978 received the title of professor in medicine.
- From 1968 till 1999 chairman of the Department of Clinical Physiology of the Medical Center of Postgraduate Education in Warsaw.
- In 1988/89 visiting scientist in National Institutes of Health, Bethesda, USA.
- Main interest: erythropoietin (early works) and experimental cardiology.
- Co-author of early papers proving that kidney is the main source of erythropoietin and establishing its gross chemical structure. Later work addressed the mechanism of excitation-contraction coupling in normal cardiac myocytes and cellular remodeling in heart hypertrophy and failure. Recently these lines of interest merged since erythropoietin has been shown to have the cardioprotective effects in heart ischemia and failure.
- At present retired professor of the Medical Center of Postgraduate Education and chairman of the Scientific Council of the Scientific Board of the National Institute of Cardiology in Warsaw.

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