Erythropoietin (EPO) is a member of the cytokine type I family and is mainly synthesised in the peritubular cells in the cortex-medullary border of the kidney. Under hypoxic conditions erythropoietin is released and increases the amount of circulating red blood cells by enhancing the development of precursor red cells and by protecting red blood cells from apoptosis. As a therapeutic agent erythropoietin became widely used in treating anaemia resulting from chronic kidney disease and myelodysplasia after chemotherapy or radiation.

The effects of EPO are mediated by a specific transmembrane EPO receptor which is expressed mainly on haematopoietic progenitor cells. EPO induces homodimerisation of the EPO receptor with subsequent activation of the Janus kinase 2, tyrosine phosphorylation of the EPO receptor and signal transducer and activator of transcription factor 5 (STAT5). This activates PIP3 kinase, Akt and MAP kinases with subsequent suppression of apoptosis.

Anti-apoptotic pathways downstream of EPOR are activated by EPO to inhibit apoptosis associated with ischemia and reperfusion injury in vitro and in vivo. It has also been hypothesised that alternative EPO binding complexes such as a heteroreceptor comprised of the EPOR and the GM-CSF/IL-3/IL-5 receptor β-common chain may mediate the cytoprotective effects of EPO.

These non-haematopoietic effects of EPO have been described in different tissues under ischemic conditions. In a rat model of focal brain ischemia, EPO administration reduced infarct size. Similarly, EPO reduced renal and myocardial injury after ischemia and reperfusion. Experimental models of acute reperfused myocardial infarction showed a decrease in infarct size, an improved cardiac contractility and better haemodynamics.

Administration of EPO was not only beneficial directly after induced myocardial infarction but even led to an improved remodelling and capillary density if given three weeks thereafter. In most of these experimental studies, EPO was given systemically and a high-dose regimen was used. Treatment duration differed from single dose administration to daily injections over a week (Table 1). Long-term application of EPO increased the haematocrit as expected, whereas short-term EPO application only increased reticulocytes without changing haemoglobin levels.
Studies using low-dose EPO therapy also led to an improved cardiac function and neovascularisation in rats after myocardial infarction without altering haematocrit.\textsuperscript{17, 23} This is particularly important as large clinical trials showed that an increase of haematocrit due to long term EPO treatment was accompanied by increased rates of thromboembolic events.\textsuperscript{25-27}

Potential mechanisms that may contribute to the cytoprotective effect of EPO include inhibition of apoptosis and improved cardiomyocyte survival since infarct size and reperfusion injuries are related to the extent of myocardial apoptosis. Functional recovery after ischemia and reperfusion was associated with a decrease in apoptotic cells and was abolished in the presence of specific inhibitors of the PI3K/Akt pathway.\textsuperscript{28, 29} Other signalling pathways involved are MAPK p38 and p42/44.\textsuperscript{30} Additional mechanisms for protection of ischemia/reperfusion injury may include attenuation of inflammatory responses\textsuperscript{31} and oxidative stress.\textsuperscript{12, 22-35}, modulation the cardiac Na+/K+ pump\textsuperscript{36} and stimulation of atrial natriuretic peptide release\textsuperscript{37} (Figure 1).

### Table 1: Effects of EPO in experimental myocardial infarction

<table>
<thead>
<tr>
<th>Animal Model</th>
<th>Species</th>
<th>EPO dosage</th>
<th>EPO application time</th>
<th>Ref.</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemia/Reperfusion</td>
<td>Rat</td>
<td>5.000 IU/kg daily for 7 days</td>
<td>18</td>
<td>Reduction in cardiomyocyte loss, normalization of hemodynamic functions within 1 week</td>
<td></td>
</tr>
<tr>
<td>Ischemia/Reperfusion</td>
<td>Rat</td>
<td>5.000 IU/kg 30 min. after ischemia</td>
<td>50</td>
<td>Reduction in infarct size</td>
<td></td>
</tr>
<tr>
<td>Ischemia/Reperfusion</td>
<td>Rat</td>
<td>5.000 IU/kg Before, at the beginning or at the end of ischemia</td>
<td>17</td>
<td>Reduction in infarct size and myocardial apoptosis</td>
<td></td>
</tr>
<tr>
<td>Ischemia/Reperfusion</td>
<td>Rabbit</td>
<td>5.000 IU/kg After ischemia</td>
<td>20</td>
<td>Improvement in myocardial function, reduction in myocardy apoptosis</td>
<td></td>
</tr>
<tr>
<td>Ischemia/Reperfusion</td>
<td>Dog</td>
<td>100-1000IU/kg After ischemia</td>
<td>29</td>
<td>Reduction in infarct size</td>
<td></td>
</tr>
<tr>
<td>Ischemia/Reperfusion</td>
<td>Rat</td>
<td>30µg/kg darbopoietin Before ischemia</td>
<td>51</td>
<td>Reduction in infarct size, improved LV EF, decrease in apoptosis</td>
<td></td>
</tr>
<tr>
<td>Permanent Occlusion</td>
<td>Rat</td>
<td>40µg/kg darbopoietin 3 weeks after occlusion</td>
<td>23</td>
<td>Improved cardiac function, progenitor cell mobilisation, increased vascularisation</td>
<td></td>
</tr>
<tr>
<td>Permanent Occlusion</td>
<td>Rat</td>
<td>5.000 IU/kg After occlusion</td>
<td>21</td>
<td>Improved survival, haemodynamic parameters, progenitor cell mobilisation, decreased apoptosis, increased vascularisation</td>
<td></td>
</tr>
<tr>
<td>Permanent Occlusion</td>
<td>Rat</td>
<td>5.000 IU/kg After occlusion</td>
<td>52</td>
<td>Reduction in apoptosis</td>
<td></td>
</tr>
<tr>
<td>Permanent Occlusion</td>
<td>Rat</td>
<td>3.000 IU/kg After occlusion</td>
<td>34</td>
<td>Reduction in infarct size, improvement LV function</td>
<td></td>
</tr>
<tr>
<td>Permanent Occlusion</td>
<td>Rat</td>
<td>40µg/kg darbopoietin After occlusion</td>
<td>53</td>
<td>Reduction in infarct size, improvement LV function, increase in capillaries</td>
<td></td>
</tr>
</tbody>
</table>

![Figure 1:](image-url)
However, recent studies question the concept of EPOR signalling in endothelial cells and cardiomyocytes since no functional EPOR was detected on these cells. Accordingly in some non-haematopoietic tissue protection models EPO was unable to preserve renal function after ischemia reperfusion injury and did not alter lipopolysaccaride-induced myocardial depression and myocyte apoptosis.

Recent studies investigating the local EPO application in myocardial ischemia revealed contradictory results. A single high-dose intramyocardial administration of EPO led to an enhanced intracardiac proliferation and improved cardiac function after permanent coronary occlusion in rats. After ischemia and reperfusion, however, a single intramyocardial dose of EPO was not sufficient to improve cardiac left ventricular ejection fraction measured in MRI. Yet, additional application with endothelial progenitor cells improved regional wall movement by MR as compared to EPC injection alone. Underlying mechanisms may include anti-apoptotic and immunomodulatory effects.

Clinical studies using erythropoietin in acute myocardial infarction based on the promising results of the experimental studies numerous prospective, randomised, clinical trials have been initiated to assess potential clinical benefits of EPO administration in patients presenting with myocardial infarction. So far five studies have been completed; three further trials are still recruiting patients. (Table 2).

One of the first trials to be completed was the Efficacy Study of Erythropoietin After Revascularization in Myocardial Infarction (RE-VIVAL 3) trial. Patients presenting with ST-elevation myocardial infarction and an onset of symptoms of less than 24 hours were treated with primary percutaneous coronary intervention (PCI). Immediately after reperfusion of the infarcted vessel, after 24 hours and after 48 hours, 3.33×10^4 IU of EPO (n=68) or placebo (n=70) was given intravenously. With a cumulative dose of 100,000 IU this was the highest dose given compared to other trials. There was no significant difference in primary endpoint defined as left ventricular ejection fraction assessed by MRI after six months (EPO 52.0±9.1%, placebo 51.8±9.3%).

### Table 2: Effects of EPO in experimental myocardial infarction

<table>
<thead>
<tr>
<th>Trial</th>
<th>Patients</th>
<th>Application Time</th>
<th>Single EPO Dose</th>
<th>Cumulative EPO Dose</th>
<th>Follow up</th>
<th>Primary Endpoint</th>
<th>Secondary Endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>REVEAL</td>
<td>138</td>
<td>&lt; 4h after PCI</td>
<td>60.000 U</td>
<td>60.000 U</td>
<td>12 weeks</td>
<td>No change in infarct size assessed by MRI</td>
<td>No change in cardiac volumes, increase adverse clinical events with EPO, increase in infarct size in pats. &gt;70yrs.</td>
</tr>
<tr>
<td>HEBE III</td>
<td>529</td>
<td>&lt; 3h after PCI</td>
<td>60.000 U</td>
<td>60.000 U</td>
<td>6 weeks</td>
<td>No Change in LVEF by Radionuclide Ventriculography</td>
<td>Lower MACE in EPO group</td>
</tr>
<tr>
<td>REVIVAL 3</td>
<td>138</td>
<td>during PCI, 24h and 48h</td>
<td>33.300 U</td>
<td>100.000 U</td>
<td>6 months</td>
<td>No change in LVEF fraction assessed by MRI</td>
<td>MACCE increased by trend in EPO group</td>
</tr>
<tr>
<td>EPOC AMI</td>
<td>35</td>
<td>after PCI, 48h and 96h</td>
<td>6.000 U</td>
<td>18.000 U</td>
<td>6 months</td>
<td>No change in infarct size assessed by SPECT</td>
<td>No change in cardiac volumes or MACE</td>
</tr>
<tr>
<td>Suh et al.</td>
<td>57</td>
<td>before PCI</td>
<td>50 U/kg</td>
<td>50 U/kg</td>
<td>4 days</td>
<td>No change in infarct size by cardiac biomarkers</td>
<td>No change in infarct size assessed by MRI</td>
</tr>
<tr>
<td>EPOMI Study</td>
<td>Recruiting</td>
<td>directly after PCI</td>
<td>1000 U/kg</td>
<td>1000 U/kg</td>
<td>3 months</td>
<td>Infarct size assessed by MRI</td>
<td>Cardiac Volumes, ejection fraction, MACE</td>
</tr>
<tr>
<td>Intra-CO-EpoMI</td>
<td>Recruiting</td>
<td>during PCI</td>
<td>150 µg</td>
<td>150 µg (Darbepoetin)</td>
<td>3 months</td>
<td>Infarct size assessed by MRI</td>
<td>Cardiac enzymes, Echocardiography</td>
</tr>
<tr>
<td>E PA M I - NONDAS</td>
<td>Recruiting</td>
<td>after PCI, 24h and 48h</td>
<td>100 or 200 IU/kg</td>
<td>300 or 600 IU/kg</td>
<td>12 months</td>
<td>Infarct size assessed by MRI and cardiac biomarkers</td>
<td>MACE</td>
</tr>
</tbody>
</table>
The cumulative six-month incidence of death, recurrent myocardial infarction, stroke or target vessel revascularisation was 13.2% in the erythropoietin group and 5.7% in the placebo group (P=0.15). The recently published HEBE III trial was the largest trial so far and enrolled 529 patients with STEMI and successful PCI. Patients were randomised to either receive standard medical care alone (n=266), or in combination with a single dose 60,000 IU of epoetin alfa (EPO n=263) within 3 h after PCI.

Left ventricular ejection fraction after six weeks assessed by planar radionuclide ventriculography was defined as primary endpoint and showed no significant difference between erythropoietin group (53±10%) and control group (52±11%; P = 0.41). However the incidence of cardiovascular events within six weeks after PCI including cardiovascular death, re-infarction, emergency re-PCI or coronary artery bypass grafting, stroke and clear symptoms of heart failure was increased in the control group as compared to the EPO group (19 vs. 8; P = 0.032).

In the Reduction of Infarct Expansion and Ventricular Remodeling With Erythropoietin After Large Myocardial Infarction (REVEL) trial, 138 patients presenting with STEMI were enrolled and randomised to either receive a single dose of 60,000 IU EPO within four hours after successful PCI or placebo. There was no significant difference concerning the primary endpoint defined as infarct size assessed by MRI after 12 weeks. In a subgroup analysis patients >70 years of EPO treatment even increased infarct size although the low patient number needs to be taken into account. EPO treatment was also associated with an increased number of adverse clinical events. After EPO four per cent of the patients underwent death, recurrent myocardial infarction, stroke or stent thrombosis, none of these events were observed after placebo.

Two smaller trials were conducted with results that were in line with these trials: In the Erythropoietin Prevention Trial of Coronary Restenosis and Cardiac Remodeling After ST-Elevated Acute Myocardial Infarction (EPOC-AMI), 35 patients presenting with STEMI were randomised to either receive 6,000 IU of EPO after PCI, at 48 and 96 hours of standard medical care. No improvement in infarct size assessed by single photon emission computed tomography (SPECT) after six months could be observed. Moreover, no significant differences in cardiac volumes or major adverse cardiac events (MACE) were observed. The study conducted by Suh et al. randomised 57 patients with STEMI either to PCI and standard medical care or to an additional single dose of 50 U/kg EPO before PCI. After four days the release of cardiac enzyme and the absolute infarct volume in MRI did not differ between two groups.

**DISCUSSION**

The clinical trials outlined above used greatly different regimens of EPO treatment, ranging from 50 IU/kg up to a cumulative dose of 100,000 IU. As compared to the experimental studies even the highest dose of EPO is at least fourfold lower of the dosages used in experimental myocardial infarction. Thus, it cannot be excluded that higher dosages or prolonged application may prove beneficial. Yet, considering the experimental data with decreases in infarct size up to 40% after EPO and efficacy of even low dose EPO, suggest that the preclinical finding of a beneficial effect of EPO in AMI does not translate into clinical practice.

Efficacy of short-term and high-dose EPO treatment in the REVIVAL-3 trial is assumed as increased levels of reticulocytes, platelets and progenitor cells were observed. With the lower EPO dose of 18,000 IU in the EPOC-AMI trial, no increase in progenitor cells or platelet count was described. Haematocrit and haemoglobin levels remained unchanged in all of the published trials. Accordingly, no higher rates in thromboembolic events during EPO treatment were observed. Nevertheless the number of patients enrolled in the clinical trials is not sufficient to fully exclude an increased thromboembolic risk, so further evaluation of EPO’s influence on platelet function and coagulation parameters in these patients may aid in determining this risk.

The performed trials showed that short-term treatment with EPO in AMI is safe, but seems to have no clinical benefit concerning improvement in myocardial function assessed by either left ventricular ejection fraction or infarct size in SPECT or MRI or reduction in infarct size. The time point for evaluation of the primary endpoint differed from four days to six months. In the HEBE III trial, the largest study with 526 patients, left ventricular ejection fraction was evaluated after six weeks using radionuclide ventriculography. The REVIVAL 3 trial had a longer follow up and analysed left ventricular ejection fraction after six months in MRI. Independent from the time point or imaging method no improvement could be observed in any study published so far.

A possible clinical benefit was observed in the HEBE III trial, where the incidence of MACE was lower in the EPO group. This was due to a higher rate of heart failure in the control group (7 vs. 1; p=0.034). In contrast, patients enrolled in the REVIVAL 3 trial had a trend towards a higher MACCE, mainly due to a higher incidence of target lesion revascularisation. Similarly the REVEL trial an increased number of adverse events was found after treatment with EPO. Considering these controversial results it has to be taken into account that these studies are underpowered for clinical endpoints.

The evidence from animal studies that erythropoietin could enhance re-endothelialisation leading to inhibition of in-stent restenosis by directly protecting endothelial apoptosis and mobilising endothelial progenitor cells was addressed in the EPOC-AMI trial. Primary endpoints included in-stent neointima volume and instant late lumen loss but showed no effect of low dose EPO treatment on neointima generation. Results of the other trials regarding neointima generation may further clarify the role of high dose EPO on neointima generation.

All trials enrolled patients whose left ventricular ejection fraction (LVEF) was mildly reduced, but kept over 50% on follow-up, suggesting that all patients were treated optimally by PCI and standard therapy. Although subgroup analysis in the REVIVAL-3 study showed no benefit of EPO treatment in patients with severely impaired LV function the number of patients may be too low to allow detecting subtle changes in LVEF. Moreover, in the current clinical setting LV function might not be the best surrogate marker to evaluate the possible beneficial effects of EPO during AMI. Since recovery of LV function in many patients with AMI occurs up to six months after the event, it would be interesting to evaluate LVEF after six months in patients in the REVEL and the HEBE III trials.

Thus, erythropoietin failed to fulfil the expectations of improving ischemia reperfusion injury that were raised in numerous experimental studies since no changes in myocardial function or infarct size were observed in five clinical trials. The increase in adverse events in two of these trials should sound a note of caution.
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