Introduction and aim of the thesis
INTRODUCTION

Heart failure (HF) is a common and complex clinical syndrome. HF is associated with very poor outcome (1-year mortality of 20%), which becomes even worse when co-morbidities are present. HF may arise from various aetiology and has variable clinical manifestations, notably including dysfunction of other organs than the heart, such as skeletal muscle, kidney and the bone marrow. In addition to increased mortality, high rates of hospital admission, extensive diagnostic testing and the chronic use of multiple pharmacological agents account for an immense healthcare expenditure. As a result, about 1% of the entire health care budget is spent on HF. Over the past decades, treatment options for HF have improved importantly with the addition of beta-blockers, angiotensin converting enzyme (ACE)-inhibitors, aldosterone antagonists, angiotensin receptor blockers (ARBs), and device therapy such as biventricular pacing with cardiac resynchronization therapy and internal defibrillators. However, despite all these treatment options, morbidity and mortality rates are still increasing globally. New therapeutic possibilities either focusing on the prevention of HF or on the improvement of signs and symptoms of already existing HF are therefore pivotal.

Before overt HF ensues, the heart, specifically the left ventricle (LV) undergoes changes in response to damaging stimuli, which is referred to as LV remodelling. Common aetiologic factors for HF are hypertension, ischemic heart disease with or without myocardial infarction (MI), valvular disease and myocarditis. All of these factors initiate the remodelling process. The initial response of the heart to stress is cardiomyocyte hypertrophy, while in later stages fibroblast and macrophage activation leads to apposition of extracellular matrix, and often remodelling becomes progressive and finally results in LV dilatation and congestive HF.

So although deemed initially useful, remodelling over time often becomes maladaptive. Maladaptive remodelling is characterised by excessive myocyte hypertrophy and apoptosis, interstitial and perivascular fibrosis (including replacement fibrosis) and a decrease in number of capillaries. The growth (in number and size) of capillaries, which theoretically would be needed to supply the hypertrophic LV with oxygen and nutrients, is insufficient in LV hypertrophy, thus creating an imbalance between the number of capillaries and cardiomyocytes. This imbalance is thought to further contribute to the remodelling process as it causes chronic hypoxia and less flow reserve, with further tissue damage as a result. Without treatment, this downward spiral continues, resulting in HF, and finally in death. Current treatment targets in HF target the cardiomyocyte hypertrophy or are anti-fibrotic. However, the microvasculature is difficult to target and no specific treatments are available.

One of the common co-morbidities in HF is anaemia, which is associated with a poor outcome. Its prevalence varies with the severity of HF and with the definition of anaemia that is used. The causes of anaemia in HF are only partially understood, although several mechanisms have been implicated, including treatment with ACE-inhibitors, a blunted erythropoietin (EPO) production due to renal dysfunction, congestion, and iron deficiency. However, these causes only partially explain the severity of anaemia and proved to be difficult targets for treatment.
EXPERIMENTAL FINDINGS AFTER EPO ADMINISTRATION

EPO is a haematopoietic hormone and increases red blood cell maturation and growth. Remarkably, EPO has extensive non-haematopoietic properties, which suggest that EPO has functions other than haematopoiesis alone. A functional EPO receptor (EPOR), which was previously thought only to be present in haematopoietic progenitor cells, is also expressed in non-haematopoietic systems, such as the cardiovascular system (cardiomyocytes, endothelial cells) and the central nervous system. These discoveries fuelled intense research on the non-haematopoietic effects of EPO. EPO modulates a broad array of cellular processes that include progenitor stem cell release, cellular integrity and apoptosis, and angiogenesis. EPO has emerged as a potential anti-apoptotic agent and a vascular growth factor with promising protective potential in the setting of acute and chronic myocardial ischemia. In numerous experimental studies it has been established that EPO exerts cytoprotective and angiogenic effects. The anti-apoptotic effect of EPO is the predominant acute protecting mechanism of EPO during ischemia, while induction of neovascularisation is the predominant long-term effect of EPO.

EPO administration in ischemia-reperfusion (I/R) injury models reduces cardiomyocyte loss by 50%, decreases infarct size, enhances LV function and reduces apoptosis, even when EPO is administered after the onset of reperfusion or in low non-haematocrit increasing dosages. In models of permanent MI, EPO induces neovascularisation, but only in the presence of ischemia. Because of these non-haematopoietic effects, EPO may potentially represent a powerful pharmacologic addendum in the fight against cardiovascular diseases.

RESULTS FROM CLINICAL TRIALS WITH EPO

The very first feasibility and safety study in patients with acute MI was performed by Lipsic et al. No adverse events were recorded during 30-days follow-up. Left ventricular ejection fraction (LVEF) measured with planar radionuclide ventriculography after four months, was similar in both groups. Although haematocrit levels were not significantly different in both groups, EPO treatment significantly increased the amount of endothelial progenitor cells (EPCs) (CD34+/CD45-). Further small studies showed variable results that ranged from no effect on enzymatic infarct size and LV function to increase in angiogenesis signalling proteins in peripheral blood mononuclear cells (table 1). One of these studies, the REVIVAL-3, raised safety concerns. The REVIVAL-3 evaluated the effect of a short-term infusion of high dose EPO in patients with ST-segment elevation MI treated with primary percutaneous coronary intervention (PCI). The primary endpoint was LVEF assessed by MRI at 6 months, which was similar in both groups. The secondary endpoint of this study, consisting of combined clinical events (death, recurrent MI, infarct-related artery revascularization, or stroke) occurred in 13% of EPO-treated patients as compared with 6% in the placebo group. Although this difference did not reach statistical significance, the tendency towards an increase in serious adverse events raised concerns about the safety profile of EPO in patients with MI.

Several small trials addressed the use of EPO in chronic HF. Again, these small scale studies
### Table 1. Clinical studies: The effects of EPO in acute myocardial infarction

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STEMI = ST-elevation myocardial infarction, NSTEMI = non-ST-elevation myocardial infarction, EPC = endothelial progenitor cell, EPO = erythropoietin, EPOR = erythropoietin receptor, MI = myocardial infarction, PBMC = peripheral blood mononuclear cell, PCI = percutaneous coronary intervention, p-PI3K = phosphorylated phosphatidylinositol 3-kinase, VEGFR-1 = vascular endothelial growth factor receptor 1, LVEF = left ventricular ejection fraction, MACE = major adverse cardiac events, LV = left ventricle.

showed variable results. Some studies showed improvement in cardiac function, less hospitalisation and enhancement of exercise capacity, where other studies showed no effects on six minutes walk test, New York Heart Association (NYHA)-class and LVEF (table 2).50-55

Large prospective, randomised, clinical trials are necessary to provide definitive answers if EPO could be beneficial as an pharmacologic addendum against cardiovascular disease.56 To evaluate the use of EPO in myocardial ischemia, our group therefore designed a multicenter, prospective, randomised, openlabel trial with blinded evaluation of the primary endpoint (HEBE III).57 The primary objective was to study the effect on LVEF of a single bolus of EPO, administered directly after a primary PCI for a first MI. Eligible patients were randomly assigned to either receive standard medical care or a single bolus with 60,000 IU of EPO on top of
standard medical care within three hours of the PCI procedure. Primary endpoint of the study was LVEF assessed by planar radionuclide ventriculography after six weeks. The results of the RED-HF trial (a large, randomised, clinical trial evaluating morbidity and mortality with the use of EPO in chronic HF) are expected at the end of 2011 and should give the definitive answer to the use of EPO in chronic HF.58
Both the REVEAL and the HEBE III study were designed to evaluate the effect of EPO in first ST-segment elevation MI.\textsuperscript{57,59} As the results of the REVEAL are still awaited, very recently the results of the HEBE III were published.\textsuperscript{60} A single dose of EPO did not improve global LVEF after six weeks. However, more major adverse cardiovascular events occurred in the control group than in the EPO group, suggesting a potentially relevant cardioprotective effect and a favourable clinical safety profile of EPO.

Recent large trials with EPO in other diseases, i.e. in patients with chronic kidney disease (CHOIR, TREATE), haemodialysis (CREATE), and stroke (German Multicenter EPO Stroke Trial) indicated that high dose of EPO is associated with excess stroke and mortality, possibly due to the hyperviscosity and hypercoaguability associated with high haematocrit.\textsuperscript{61-64} We therefore have postulated that EPO should be administered in low dosages,\textsuperscript{42} so that the undesired side effects due to increasing haematocrit are avoided, yet the desirable anti-ischemic and cytoprotective effects are preserved. Alternative EPO-like compounds, which have no effects on erythropoiesis, have been advocated in this respect.\textsuperscript{65-68}

**Figure 1.** Figure shows the mechanisms of the various factors described in this thesis and its interplay with ischemia and left ventricular hypertrophy. HIF = hypoxia inducible factor, EPO = erythropoietin, EPOR = EPO receptor, HO-1 heme oxygenase-1, CO = carbon monoxide, VEGF = vascular endothelial growth factor, EPC = endothelial progenitor cell. For colour figure, see supplement 1.
AIM OF THIS THESIS

The aim of this thesis was to evaluate in HF the non-haematopoietic effects of EPO and associated factors with similar protective mechanisms, such as estradiol and heme arginate. In the first part of this thesis, we evaluated the extra-erythropoietic mechanisms of EPO and its role in the EPO-EPOR system. In chapter 2, we review the function of EPO and its receptor in the cardiovascular system and the use of EPO in both acute MI as in chronic HF. In chapter 3 we evaluate the mechanisms of EPO-induced vascular endothelial growth factor (VEGF) production in the heart and establish that VEGF is crucial for EPO-induced improvement of cardiac performance. In chapter 4, the role of the EPOR in physiologic (cardiac) hypertrophy is investigated. We show that EPO-EPOR signalling is crucial for physiologic hypertrophy. Chapter 5 describes the existence of I/R injury in ventricular tissue during coronary artery bypass graft surgery with the use of cardiopulmonary bypass. It shows that ventricular tissue maybe more sensitive to detect changes than atrial tissue. Furthermore, this chapter also includes the design and rationale to further study these mechanisms.

The second part of this thesis evaluates interventions (with estradiol and heme arginate) in downstream pathways of EPO and focuses on feasible targets. These factors were chosen as they share working mechanisms with EPO, namely anti-apoptotic and angiogenic properties. In chapter 6, we investigate whether estradiol administration could induce EPC-mediated neovascularisation in a model of hind-limb ischemia. In chapter 7 we focus on the use of heme arginate as a reactive oxygen species scavenger in cardiac I/R injury. Chapter 8 evaluates the existence of HF-associated anaemia in an experimental HF model and if EPO administration could be an efficacious treatment option.
Chapter 1

REFERENCES


Chapter 1

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Chapter 1


52. Reduction of infarct expansion and ventricular remodeling with erythropoietin after large myocardial infarction (REVEAL). 2009;


PART 1

Mechanistic insights in the extra-erythropoietic mechanisms of erythropoietin