The effect of angiotensin (1-7) on bone marrow stem cells
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Chapter 1

Introduction and aim of the thesis
Current therapeutic approaches to chronic heart failure (CHF) entail pharmacological reductions in oxygen demand in tissue with insufficient vascular supply or interventional restoration of blood flow. Pharmacologically induced reductions in energy needs or ventricular remodeling, although potentially beneficial for cardiac function, have hardly affected prognosis, as mortality rate in patients with CHF has remained stable over the last 5 years [1]. In addition, mechanical revascularization carries the risk of restenosis. Alternate therapeutic approaches would therefore be desirable in patients with CHF. Accordingly, investigations have focused on the development of “stem-cell based regenerative medicine” as potential alternative.

1.1 Chronic heart failure
Chronic heart failure (CHF) is clinical syndrome which may develop as a consequence of both peripheral and cardiac disorders characterized by various clinical signs and symptoms. CHF is a progressive disorder of neurohormonal activation and left ventricular remodeling. After acute myocardial infarction, all structural alterations in cardiac architecture, at organ as well as at tissue and cellular level, are referred to as myocardial remodeling. Post-infarction remodeling has been divided into two phases: early compensatory phase and late decompensate phase [2,3]. During the remodeling process three major components of the myocardium are involved in: myocytes, extracellular matrix and blood vessels. In the early phase irreversibly damaged tissue is replaced by scar tissue: in the infarcted area, myocyte necrosis and acute permeable inflammation at the border of the necrotic myocytes occurs, followed by inflammation, fibroblast and vascular cell proliferation, extracellular matrix deposition, as well as circulating or bone marrow stem cell recruitment. In the non-infarcted area, to compensate for the loss of contractile tissue, myocytes hypertrophy and interstitial fibrosis occur. During the development of cardiac hypertrophy, a mismatch between the number of capillaries and the size of cardiomyocytes develops, leading to myocardial hypoxia [4,5]. The putative capability of angiogenic stem cell is to generate neovascularization via vasculogenesis. Hence, it has been postulated that therapeutic transplantation of bone marrow angiogenic stem cells may contribute to postinfarcted neovasculogenesis in the myocardial tissue, either by paracrine or regenerative mechanisms.

1.2 Formation of blood vessel growth
All blood vessels share a number of basic features, although detailed gene expression pattern, morphology, and function may vary between different vessel beds. The inside of blood vessels is lined with endothelium, consisting of a layer of endothelial cell, which separates blood from tissues. The outside of the endothelium is covered with a specialized layer of connective tissue followed by a layer of mural cells (pericytes and vascular smooth muscle cells) [6,7]. Vessel growth requires tip cells serving as pioneer guider and pericytes to stabilize the vessel. On Matrigel coated culture plates, in-vitro culture with bone marrow-derived angiogenic cells mimics the in-vivo vessel growth (Figure 1). Neovascularization is a
tightly controlled process where endothelial/progenitor cell proliferation and migration are regulated by secreted factors as well as by surrounding cells and matrix [8]. The vascular system mainly originates from two fundamental processes: vasculogenesis and angiogenesis. Vasculogenesis is the formation of a primary plexus of vessels by stem cells, so called hemangioblasts. During this procedure, endothelial precursors undergo differentiation to endothelial cell in combination with the formation of an in-situ vascular network and remodeling, whereas angiogenesis is defined as the sprouting of new capillaries or splicing from preexisting vessels [9,10]. It has long been accepted that vasculogenesis is limited to early embryo period but not in adulthood, while angiogenesis occurs in both embryonic and postnatal life. However, this paradigm has been changed now that both vessel formations are observed during embryonic and adult growth processes [11-13]. After ischemic injury, ischemia leads to expression of hypoxia inducible factor-1 alpha; stromal derived factor-1; and the pro-angiogenic factor-vascular endothelial growth factor, which initiates the procedure of neovascularization characterized by at least four steps; i.e. mobilization; permeability; proliferation and lumen stabilization [8,14].

**Figure 1:** Vascular tube formation on Matrigel: after cultured with EBM-2 medium for 7 days, bone marrow-derived endothelial progenitor cells were reseeded on Matrigel coated culture plates. Vascular tube formation was observed within 6 hours and can be further expanded after 24 hours culture. The vascular tube formation may be in network form or vascular tree-like structure (left). In this structure, tip cell served as pioneer cell (right upper) to open the avenue and guide the following endothelial cells or to split the branches (right lower). Arrow shows the tip cell with filopodia.

1.3 Angiotensin-(1-7) and its pleiotropic actions
The renin-angiotensin-system (RAS) is a cascade of enzymatic reactions resulting ultimately in the formation of angiotensin II. Recent research has expanded the knowledge about the RAS by adding new components to the pathways: angiotensin-(1-5), angiotensin-(1-7) [Ang-(1-7)], angiotensin-(1-9), an angiotensin converting enzyme (ACE) homologous enzyme,
ACE2, and the G-protein-coupled receptor Mas as a molecular receptor for Ang-(1-7) [15-17]. Ang-(1-7) can be directly or indirectly converted from Ang I or Ang II by several enzymes (Figure 2). After cutting of the last amino acid-phenylalanine, Ang II is covert to Ang-(1-7) (Figure 3). As a counteractor of angiotensin II, it has become clear that Ang-(1-7) contributes to the cardiovascular effects of ACE inhibitors and AT$_1$-receptor-blockers both in experimental conditions and human [18,19].

One of the major understandings of the role of Ang-(1-7) is related to cardiac effects via Mas receptors. In a rat myocardial infarction model, chronic intravenous infusion of Ang-(1-7) markedly attenuated heart failure and reduced myocardial infarction [20]. An important aspect related to the cardioprotection produced by the Ang-(1-7)-Mas axis lies on its independent role on blood pressure [21-23]. In addition to its effects on cardiac remodelling, Ang-(1-7) possesses anti-arrhythmic effects, which may be due to changes in sodium pump activity [24]. Moreover, Ang-(1-7) mediated by Mas receptors, appears to be involved in antiproliferation of vascular smooth muscle cell, fibroblast and in-stent induced neointima formation [25-27].

**Figure 2:** The formation of Angiotensin-(1-7) and its counteractor effects against Angiotensin II in cardiovascular system. The number between brackets indicates the composition of acid amino peptides. In the RAS, Ang-(1-7) can be directly converted from Ang I via endopeptidases or from Ang II via angiotensin converse enzyme-2 (ACE$_2$), proslylendopeptidase (PEP) and proslylcarboxypeptidase (PCP). Furthermore, ACE$_2$ can also form Ang-(1-9), from which Ang-(1-7) can be converted via ACE and neutral endopeptidase (NEP).
It is well documented that the vasodilatory activity of Ang-(1-7) is endothelium dependent [28]. By binding to endothelial cells Ang-(1-7) can stimulate the production of nitric oxide, prostaglandins or endothelium-derived relaxing factor [29]. Importantly, Ang-(1-7) induces the release of nitric oxide through coordinated phosphorylation or dephosphorylation of endothelial nitric oxide synthase. This effect involved an upstream mechanism; the activation of the phosphatidylinositol 3-kinase (PI3k)-Akt pathway [30], which may play a crucial role in differentiation of bone marrow-derived stem cells. Recently, an increasing body of evidence from Rodgers’s group showed that Ang-(1-7) administration significantly improved bone marrow recovery after radiochemotherapy in cancer patients [31-34], supporting a role for Ang-(1-7) in bone marrow cell related processes. The effects of Ang-(1-7) on bone marrow derived endothelial progenitor cells will be studied in the present thesis.

1.4 The aim of this thesis
In the last decade, stem cell therapy displayed a promising strategy for the treatment of CHF. Transplantation of angiogenic bone marrow-derived endothelial progenitor cells (BM-EPCs), however, generated inconsistent results both in animal experiments and clinical trials. Moreover, the functional activity of EPCs is impaired in patients with CHF [35,36], which may negatively influence therapeutic potential of autologous bone marrow cells. Early pharmacological intervention may salvage the damaged heart and improve the function of autologous bone marrow stem cells. Therefore, the aim of this thesis was to investigate whether pharmacological pretreatment of BM-EPCs with Ang-(1-7) may enhance therapeutic angiogenesis in the failing heart. The relevant experiments for this investigation were conducted in three steps:
1. The effects of Ang-(1-7) on bone marrow-derived progenitors were extensively evaluated both in vitro and in vivo.
2. A promising technique was set up to homogenously transplant bone marrow-derived angiogenic cells into the rat heart via intracoronary delivery.
3. Therapeutic potential of bone marrow-derived progenitors was evaluated in a rat myocardial infarction model. The effect of Ang-(1-7) on bone marrow progenitors, including hematopoietic and angiogenic progenitors, was comprehensively addressed in Chapter 2. In Chapter 3, a technique was established to transplant BM-EPCs into rat heart via intracoronary delivery. Then, based on this technique, therapeutic potential of ex-vivo expanded bone marrow-derived angiogenic cells was evaluated in a rat myocardial infarction model (Chapter 4), also addressing the question whether in vitro pre-stimulation of bone marrow-derived angiogenic cells by Ang-(1-7) could further enhance therapeutic potential of these cells. Chapter 5 presents a detailed review, describing the microenvironment of BM-EPCs in response to ischemia injury and physiological and pharmacological factors beneficial to BM-EPCs function, as well as future perspectives for cell based therapeutic angiogenesis. Finally, the results of these studies were discussed and summarized in Chapter 6.
INTRODUCTION AND AIM OF THE THESIS

Reference List


