Stem cell-mediated regeneration of the infarcted heart

Velde, Susanne van der

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Chapter 1

Introduction to the thesis

Susanne Vandervelde
Introduction
Myocardial infarction (MI) is one of the major health threats of the Western civilized world. In 2003 in the Netherlands 34% of all deaths were due to cardiovascular disease, of which approximately 32.5% is related to ischemic heart disease [1]. Although acute mortality post-MI subsides because of early interventions, late mortality due to heart failure increases. There is still no definite cure to functionally restore heart tissue that suffered from infarction. Although medication to endure life exists, conventional medical management does not correct the underlying defects.

Myocardial infarction occurs as the consequence of occlusion of one of the branches of the coronary artery system. The coronary vascular system functions as main supplier of oxygen and nutrition for the heart tissue, and as transportation system for waste products from the underlying heart tissue, i.e. the myocardium. Once a coronary artery occludes, the downstream myocardium is deprived of oxygen and nutrition, which results in myocardial ischemia (Fig 1). Prolonged ischemia results in the death of heart muscle cells, the cardiomyocytes.

Unfortunately, most of the cardiomyocytes, if not all, are terminally differentiated cells, that are not capable of proliferation to repair the defect. In the subsequent remodeling phase, dead cardiomyocytes are removed and structural integrity is maintained by formation of collagenous scar tissue. However, scar tissue does not contract, which, in time, causes the cardiac pump function to deteriorate. At first, the remaining healthy myocardium attempts to compensate for this loss of contractility by growth of the spared cardiomyocytes, i.e. cardiac hypertrophy. However, the remaining cardiomyocytes cannot sustain this overactive state and further deterioration of cardiac function is inevitable. Also, the lumen of the heart becomes dilated and adequate cardiac function is further hampered. This process, called heart failure, will eventually be a cause of death for many patients suffering from ischemic heart disease.

To put an end to this negative spiral of loss of cardiac function, the myocardium should be restored in an early phase post-MI. Interventions that aim for early cardiac regeneration
need to prevent scar formation, i.e. need to enable the development of new myocardium. This generated myocardium would comprise cardiomyocytes and vasculature in a tightly controlled extracellular matrix that forms a continuum with the spared myocardium. Ideal candidates for regeneration are stem cells. Stem cells are defined as cells that can self-renew *ad infinitum* and that can differentiate into various lineages and cell types [2]. According to this definition, stem cells should be able to build new myocardium comprised of cardiomyocytes, vascular endothelial cells and other supporting cells. Moreover, since stem cells can self renew indefinitely, a few stem cells should suffice for adequate regeneration. Stem cell-mediated cardiac repair is no sinecure yet and, although research started little over a decade ago, it is still in its infancy.

In the nineties of the previous century investigations on regeneration of myocardium started with studies on repopulation of the ischemic cardiac muscle by (younger) healthy cells, not necessarily multipotent stem cells [3]. First, regenerative research of the heart was focussed on the potency of fetal and neonatal cardiomyocytes. Although many of these studies showed that these cells can functionally integrate en enhance recipient cardiac function [4,5], neonatal and fetal cardiomyocytes are difficult to obtain, may provoke an immunological response by the allogenic recipient and are, of course, ethically charged. Therefore, myoblasts, i.e. precursors of skeletal muscle cells, seemed a good alternative. Myoblast are autologous, abundantly present in skeletal muscle, easy to obtain, and they expand rigorous in culture. First myoblast transplantations in animal MI models seemed successful regarding incorporated cells and improvement of cardiac post-MI function [6,7]. However, initial enthusiasm was hampered when 4 of 10 patients enrolled in a phase-I clinical trial developed ventricular tachycardia after receiving autologous myoblasts intramyocardially, most likely because of electrically uncoupled transplanted muscle cells within the myocardium[8]. The search for new cells that could not only form electronically integrated cardiomyocytes, but also contribute to neovasculature, in other words, more potent cells, had begun.

In the 1960s McCulloch et al. discovered a single precursor cell that is capable of both extensive self-renewal and multi-lineage differentiation contributing to the hematopoietic system within the bone marrow, the hematopoietic stem cell (HSC) [9]. In the late nineties reports emerged that HSC are not only able to differentiate into blood cells, but can also generate muscle, liver and brain cells [10-12]. In 2001, Orlic et al. reported the first intracardiac transplantation of bone-marrow derived HSCs post-MI, which results were promising [13]. They showed that within 9 days after intramyocardial transplantation in the border of the infarcted region, 68% of the infarcted ventricle was occupied by newly formed myocardium, comprised of both proliferating cardiomyocytes and vascular structures. This remarkable and very promising paper set the tone for cardiac regenerative research and was followed by burst of scientific papers on intramyocardial injection of bone marrow-derived stem cells, which we will not enlist here. Many of them claim improved cardiac function and attenuation of adverse remodeling. However, the methods used in stem cell transplantation studies are varying. Different populations of stem cells were used in various numbers at different time points after ischemia in different infarction models in several mammalian species. Nevertheless, in stem cell-mediated cardiac regeneration, three recurrent fundamental components can be recognized: 1) the bone marrow as major stem cell reservoir, 2) the peripheral circulation as transport way of stem cells and stem cell-related signalling factors, and 3) the recipient post-MI cardiac environment as place
of repair (Fig. 2).

Our focus within stem cell-mediated cardiac regeneration research is primarily on the post-MI recipient cardiac environment. Importantly, the recipient environment is determinative for the fate of (transplanted) stem cells. To be successful, stem cell-therapy depends on a damaged but yet inviting cardiac environment that promotes homing, incorporation, survival, proliferation, differentiation and maturation of stem cells. After MI there is a vigorous inflammatory response in the infarcted area that ultimately leads to scar formation. Within this early turbulent phase post-MI stem cells need to function optimally. Surrounded by inflammatory cells and signaling factors, they need to switch fate from formation of scar tissue to restoration of adequate functional myocardium. They need to operate in a small time window to prevent deterioration of function by scar formation. Therefore, the post-MI inflammatory environment is determinative for stem cell-based regenerative therapies. In this thesis we focus on inflammation early post-MI and its effects on stem cell-mediated cardiac regeneration.

**Aim of the thesis**

*The aim of this thesis is to understand the role of inflammation in stem cell-mediated cardiac regeneration.*

The research for this thesis started with a comparison between post-MI intramyocardially injected CD133 progenitor cells and skeletal myoblasts in a rodent model of MI (Chapter 2). Although we observed comparable functional improvement, one month after injection we could not detect a single cell of the 500,000 intramyocardial engrafted CD133+ cells by immunohistological stainings. Also others have reported that the number of stem cells that could be detected in the infarcted area after cell transplantation was very low compared...
to initial engrafted amounts. This led us to the assumption that the inflammatory response post-MI might interfere with the incorporation of intramyocardially engrafted stem cells. In chapter 3 we studied the inflammatory response following myocardial infarction in two mouse models, i.e. a model of permanent ligation of a coronary artery and a model in which cryoinjury is applied to the left ventricle. In chapter 4 we compared the inflammatory and functional response of another myocardial infarction model, the reperfusion model in which the coronary artery is occluded for 30 minutes, with the permanent ligation model. In the review in chapter 5, we discuss the key signaling factors (i.e. cytokines, chemokines and growth factors), that are involved in orchestrating the stem cell driven repair processes of mobilization from the bone marrow, incorporation in the ischemic cardiac environment, differentiation into different myocardial cells, and survival and proliferation. In chapter 6 we explored the expression in the infarcted heart of the key signaling factors, i.e. inflammatory, stem cell-related and angiogenic factors, that are involved in stem cell-mediated cardiac regeneration. In chapter 7 we investigated CD14+ monocytes, that according to various studies can become endothelial progenitor cells (EPC) [14-16]. EPCs are defined as circulating BM-derived cells that contribute to neovascularization by re-endothelialization of injured vasculature. Interestingly, these monocytes are also known as macrophage progenitors, which are, of course renowned for their role in inflammation. In this study we investigated and defined this differentiation capacity of human CD14+ cells into both endothelial-like cells and macrophages. Moreover, we also tested whether this differentiation potential is altered in patients with unstable angina pectoris compared to stable angina pectoris. Angina pectoris patients have symptomatic atherosclerotic plaques within the coronary arteries, in which both macrophages and endothelial cells play a central role. In chapter 8, the observations described in this thesis are summarized and future perspectives of stem cell-mediated cardiac regeneration are discussed.

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