General discussion

Cardiovascular disease is a leading cause of morbidity and mortality in the western world. Acute myocardial infarction (AMI) is an important contributor to this cause since it induces apoptosis, necrosis and inflammation in the myocardium, resulting in a loss of cardiomyocytes.[1,2] As spontaneous regeneration of cardiomyocytes is limited, injured myocardium is replaced by non-contractile scar tissue, which leads to adverse remodelling of the left ventricle and can result in heart failure.[2,3] Therapy for AMI focuses on restoration of reperfusion and on prevention of remodeling and secondary cardiovascular events via specific drug therapy.[4,5] Although these strategies significantly reduce mortality,[6,7] they do not replace lost cardiomyocytes. The ideal AMI therapy would therefore be to stimulate revascularization of the ischemic region, to minimize the loss of cardiomyocytes, thus limiting scar tissue formation, and to replace lost cardiomyocytes.[5] New therapies for AMI, focusing on inflammatory inhibitors and stem cell therapy using adipose derived stem cells, are the subject of this thesis.

Inflammatory inhibitors

The major goal of current AMI treatment is restoration of reperfusion in the ischemic myocardium. Reperfusion, however, results in the induction of inflammation in the heart (figure 1). Through the binding of inflammatory mediators on injured and dead cardiomyocytes, inflammatory cells, especially neutrophilic granulocytes and macrophages are attracted, subsequently clearing injured cardiomyocytes from the heart. However, this inflammatory reaction induces additional damage to the myocardium.[8-10] After AMI, also non-lethal damaged cardiomyocytes lose their membrane phospholipid asymmetry.[11] As a result, the anionic phospholipids phosphatidylserine and phosphatidylethanolamine, which are normally kept within the inner plasma membrane leaflet, are then exposed in the outer membrane leaflet, a process known as ‘flip-flop’. [12] It has shown that this flip-flop can be a reversible phenomenon.[13,14] However, during reperfusion also these flip-flopped/reversibly damaged cells are targeted by inflammatory mediators, resulting in cell death of these cardiomyocytes, thereby increasing infarct size in time. A crucial mediator herein is the acute phase protein type IIA secretory-type phospholipase A$_2$ (sPLA$_2$-IIA), which was shown to bind to and to induce cell death of these reversibly damaged cardiomyocytes.[13,15,16] As depicted in figure 1, sPLA$_2$-IIA can not only induce cell death via CRP and complement binding/activation, but also via a direct cytotoxic effect, independent of other inflammatory mediators.[13] Inhibition of this inflammation cascade might therefore form a potent therapy to reduce cell death of cardiomyocytes after AMI. To this purpose we analyzed the effect of the sPLA$_2$-IIA inhibitor PX-18 on cell death after AMI. We then found in a rat model of AMI, that treatment with PX-18 significantly reduced infarct size and mortality, without impairing wound healing.[17] The latest is of utmost importance since the inflammation response itself is also essential for the healing process of the heart after myocardial infarction.[15] When using PX-18, CRP may still bind to necrotic cardiomyocytes independent of sPLA$_2$-IIA, meaning that necrotic tissue can be cleared via the CRP-complement dependent cascade (Figure 1).[9,13] Interestingly, we also found in vitro that PX-18 reduced membrane flip-flop and apoptosis in cardiomyocytes subjected to metabolic inhibition in the
absence of sPLA₂-IIA, suggesting an additional cell protective effect of PX-18 independent of sPLA₂-IIA.[17] Finally we found that after treatment with PX-18, the numbers of so called late type macrophages in the heart were significantly increased after AMI.[17] The function of macrophages in wound healing after AMI is quite diverse, including digestion of damaged tissue, but also inhibition of inflammation and promotion of regeneration.[18,19] It has been shown that early type macrophages (type 1) digest damaged tissue, while late type macrophages (type 2) promote healing.[19,20] Therefore the finding that these late type macrophages were increased after treatment with PX-18 suggests another mechanism through which PX-18 exerts a protective effect (Figure 1). Taken together, PX-18 can exert a cardiac protective effect on at least 4 different levels: (1) PX-18 inhibits membrane flip-flop, and thus prevents the pro-inflammatory state of the plasma membrane of cardiomyocytes. (2) PX-18 inhibits sPLA₂-IIA activity, and thus prohibits sPLA₂-IIA mediated cell death of at that moment reversibly damaged cardiomyocytes. (3) PX-18 directly inhibits apoptosis of cardiomyocytes. (4) PX-18 attracts late type macrophages which improve wound healing. (Figure 1)

Another inflammatory inhibitor we have studied in this thesis was clusterin. Clusterin is not only an inhibitor of complement, but also protects cardiomyocytes after ischemia in vitro, independent of complement.[21] We have now shown in a rat model of AMI, that treatment with clusterin significantly reduced infarct size and mortality, as compared to untreated rats, again without impairing wound healing.[22] The exact mechanism through which clusterin protects cardiomyocytes after AMI, next to complement inhibition, is however not known. It has been shown that during oxidative stress clusterin exerts a protective effect in H9c2 cells (a cardiomyoblast cell-line) via the Akt/GSK-3β signaling pathway.[28] It also has been suggested that clusterin plays a role in membrane recycling of cardiomyocytes in response to ischemia,[21,23-25] and that it functions as a secreted heat-shock protein or chaperone molecule. As such it has an active role in the removal of damaged cells or toxic molecules derived from (cardiac) cells.[25-27] The cytoprotective effect of clusterin has been related to its receptor megalin.[29,30] Although we have shown that megalin is expressed in cardiomyocytes of the human heart, the cell protective effect of clusterin was independent of megalin, at least in vitro after metabolic inhibition, which is in line with findings of Jun et al.[22,28] We now have also shown that after treatment with clusterin, the number of late type macrophages was significantly increased after AMI. Taken together, also clusterin forms a potential therapeutic agent in the treatment of AMI.

**Figure 1. In vivo mechanisms of cell death after AMI: the effect of PX-18 and clusterin hereon.** Ischemia induces reversible and irreversible membrane flip-flop in cardiomyocytes. During the subsequent inflammatory reaction, sPLA₂-IIA binds to flip-flopped membranes and induces cell-death, resulting in necrosis of both reversibly as irreversibly damaged cardiomyocytes. PX-18 (red) can prevent cell death of reversibly flip-flopped cardiomyocytes by inhibiting flip-flop, by inhibiting direct cytotoxic effects of sPLA₂-IIA, and also by inhibiting sPLA₂-IIA-facilitated binding of CRP and complement. Importantly, irreversibly damaged cardiomyocytes can still be cleared due to binding of CRP to these cells independent of sPLA₂-IIA. Clusterin (blue) prevents cell death by inhibition of complement, and by inhibition of flip-flop independent of the clusterin receptor megalin. In addition both PX-18 and clusterin induce macrophage infiltration, therewith improving cardiac remodeling. C: complement, CRP: C-reactive protein, †: cell death, ↓: inhibition, ○: hydrophobic phospholipids (phosphatidylcholine) that in normal cells composes most of the outer leaflet of plasma membranes, •: anionic phospholipids phosphatidylserine (PS) and phosphatidylethanolamine (PE) that are normally kept within the inner leaflet of the plasma membrane.
Changes of the plasma membrane of cardiomyocytes
during ischemia/reperfusion induced inflammation

Figure 1.
Next, an important topic is the optimal timeframe to apply these inflammatory inhibitors. We have found that application of a single bolus of clusterin as well as PX-18 directly after reperfusion did not result in a significant reduction of infarct size. Reduction was only found when the inflammatory inhibitors were administered repeatedly during the first 3 days after infarct induction.[17,22] These results therefore indicate that the acute inflammation period after AMI plays a major role in cell death induction after AMI, in addition to ischaemia itself. In conclusion, inhibition of the inflammatory response post AMI, by administration of PX-18 and clusterin, forms a new promising therapeutic approach to decrease cell death after AMI, thus preventing the development of heart failure.

Adipose derived stem cells

Although the above described inflammatory inhibitors significantly reduced infarct size, they do not replace lost cardiomyocytes. Stem cells, however, can replace lost cardiomyocytes, although results of early clinical trials using stem cell therapy in AMI are disappointing. As such, stem cell therapy still needs to be further optimized. This we have studied in the second part of this thesis, using the relatively new and promising adipose derived stem cells (ASCs). ASCs are derived from the stromal vascular fraction (SVF) of adipose tissue, and are suggested to have a high potential for the treatment of AMI.[18,32-34] ASCs can be easily harvested and have properties similar to bone marrow mesenchymal stem cells including the capacity to differentiate towards several cell types, amongst which cardiomyocytes.[18,32-35] Even more, when compared to bone marrow, adipose tissue provides up to 100 times more mesenchymal stem cells per gram tissue.[33,35] Meanwhile, several experimental studies have been performed analyzing the role of ASCs in AMI in both small and large animal models (table 1). Also the first clinical trial using ASCs to treat AMI has started recently in the Netherlands.[36]

Although the results of these animal studies generally are promising, it was also found that due to the harsh ischemic and inflammatory environment in which the cells are transplanted after AMI, massive cell death of stem cells occurs, lowering the effectiveness of this therapy.[2,37] Several factors play an important role herein, including the time point of administration, the environment of the infarcted heart, the passage of ASCs used, and its administration route (figure 2).

Time point of administration

In most animal studies investigators applied stem cells during the same operational procedure as the infarct induction itself (table 1). However, it is known that the environment in the heart changes dramatically after AMI. Massive myocardial necrosis and leukocyte infiltration might then harm survival of implanted stem cells, and therewith reduce the effectiveness of stem cell therapy.[2,38,39] Therefore, the time point of administration might significantly affect stem cell survival after AMI. Several studies have additionally investigated the time frame to apply stem cell therapy post AMI, although this was not studied directly using ASCs.[38,40-42] These \textit{in vitro} and \textit{in vivo} studies suggested that the optimal timing for stem cell therapy is subsequent to the acute inflammatory period (>3 days post AMI), but before two weeks post AMI when scar tissue is formed and regeneration capacity is limited.[42] Interestingly we found for ASCs that adhesion, proliferation and differentiation \textit{in vitro} was improved by the extracellular
Table 1: studies on ASC transplantation after AMI in coronary ligation models

<table>
<thead>
<tr>
<th>Study</th>
<th>Animal model</th>
<th>Reperfusion</th>
<th>Time after AMI</th>
<th>passage route</th>
<th>Infarctsize</th>
<th>LVEF</th>
<th>capillaries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schenke-Layland 2009 [45]</td>
<td>Lewis</td>
<td>yes</td>
<td>&lt;1 h</td>
<td>SVF</td>
<td>I.M. ↓ ns</td>
<td>↑ **</td>
<td>↑ **</td>
</tr>
<tr>
<td>Li 2011 [46]</td>
<td>Rat (a-thymic)</td>
<td>&lt;1 h</td>
<td>p2-30</td>
<td>I.M. ↓*</td>
<td>↑* ↑*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Li 2007 [47]</td>
<td>Sprague-Dawley</td>
<td>&lt;1 h</td>
<td>p3</td>
<td>I.M. ?</td>
<td>↑** ↑**</td>
<td></td>
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<tr>
<td>Yamada 2006 [48]</td>
<td>Sprague-Dawley</td>
<td>&lt;1 h</td>
<td>?</td>
<td>I.M. ↓ ns</td>
<td>↑**</td>
<td></td>
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</tr>
<tr>
<td>Hong 2011 [49]</td>
<td>Rat (a-thymic)</td>
<td>1 h</td>
<td>p3-4</td>
<td>I.M. ↓***</td>
<td>↑*** ↑**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cai 2009 [50]</td>
<td>Rat (a-thymic)</td>
<td>1 h</td>
<td>p3</td>
<td>I.M. ↓*</td>
<td>↑* ↑*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shi 2011 [51]</td>
<td>Lewis</td>
<td>1 h</td>
<td>p3</td>
<td>I.M. =</td>
<td>=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>van Dijk 2011 [52]</td>
<td>Wistar</td>
<td>yes</td>
<td>1 day</td>
<td>SVF</td>
<td>I.V. ↓ ns</td>
<td>↑ ns</td>
<td>↑ ns</td>
</tr>
<tr>
<td>Danoviz 2010 [53]</td>
<td>Lewis</td>
<td>yes</td>
<td>1 day</td>
<td>p3-4</td>
<td>T.E. ?</td>
<td>↑*</td>
<td>↑*</td>
</tr>
<tr>
<td>Zhu 2009 [54]</td>
<td>Sprague-Dawley</td>
<td>1 day</td>
<td>p5</td>
<td>I.V. ↓*</td>
<td>↑* ↑*</td>
<td></td>
<td></td>
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<tr>
<td>van Dijk 2011 [52]</td>
<td>Wistar</td>
<td>yes</td>
<td>7 days</td>
<td>SVF</td>
<td>I.V. ↓*</td>
<td>↑ ns</td>
<td>↑ ns</td>
</tr>
<tr>
<td>van Dijk 2011 [52]</td>
<td>Wistar</td>
<td>yes</td>
<td>7 days</td>
<td>p3</td>
<td>I.V. ↓*</td>
<td>↑ ns</td>
<td>↑ ns</td>
</tr>
<tr>
<td>Wang 2009 [55]</td>
<td>Lewis</td>
<td>7 days</td>
<td>p3-5</td>
<td>I.M. ↓*</td>
<td>↑* ↑*</td>
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<td></td>
</tr>
<tr>
<td>Bayes-Genis 2010 [56]</td>
<td>Rat (SCID)</td>
<td>7 days</td>
<td>p2-5</td>
<td>I.M. ↓*</td>
<td>↑* ↑*</td>
<td></td>
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<tr>
<td>Hwangbo 2010 [57]</td>
<td>Sprague-Dawley</td>
<td>7 days</td>
<td>p3-5</td>
<td>I.M. ?</td>
<td>↑* ↑ ns</td>
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<td></td>
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<tr>
<td>Berardi 2011 [58]</td>
<td>Wistar</td>
<td>8 days</td>
<td>p5</td>
<td>I.M. ?</td>
<td>= ↑ ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mouse</strong></td>
<td></td>
<td></td>
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<tr>
<td>Yu 2010 [59]</td>
<td>C57BL/6</td>
<td>&lt;1 h</td>
<td>p2-3</td>
<td>I.M. ↓*</td>
<td>↑* ↑*</td>
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<tr>
<td>Tokunaga 2010 [60]</td>
<td>C57BL/6</td>
<td>&lt;1 h</td>
<td>p3-5</td>
<td>I.M. ↓ ns</td>
<td>↑ ns</td>
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<tr>
<td>van der Bort 2009 [61]</td>
<td>FVB</td>
<td>&lt;1 h</td>
<td>?</td>
<td>I.M. =</td>
<td>↑ ns ?</td>
<td></td>
<td></td>
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<tr>
<td>Leobon 2009 [62]</td>
<td>C57BL/6</td>
<td>&lt;1 h</td>
<td>?</td>
<td>I.M. ?</td>
<td>↑* ↑*</td>
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<tr>
<td>Bayes-Genis 2010 [56]</td>
<td>NIH-Foxn1nu</td>
<td>&lt;1 h</td>
<td>p2-5</td>
<td>I.M. ↓*</td>
<td>↑* ↑*</td>
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<tr>
<td>Bai 2010 [63]</td>
<td>Mouse (SCID)</td>
<td>&lt;1 h</td>
<td>SVF</td>
<td>I.M. ?</td>
<td>↑** ↑**</td>
<td></td>
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<tr>
<td>Bai 2010 [63]</td>
<td>Mouse (SCID)</td>
<td>&lt;1 h</td>
<td>p3</td>
<td>I.M. ?</td>
<td>↑** ↑**</td>
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<td><strong>Pig</strong></td>
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<tr>
<td>Alt 2010 [64]</td>
<td>Pig</td>
<td>yes</td>
<td>&lt;1 h</td>
<td>SVF</td>
<td>I.C. ↓*</td>
<td>↑*</td>
<td>↑*</td>
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<tr>
<td>Valina 2007 [65]</td>
<td>Pig</td>
<td>yes</td>
<td>&lt;1 h</td>
<td>p3</td>
<td>I.C. ↓ ns</td>
<td>↑*</td>
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<tr>
<td>De Siena 2010 [66]</td>
<td>Pig</td>
<td>&lt;1 h</td>
<td>p5-8</td>
<td>I.C. ↓*</td>
<td>↑* ↑ ns</td>
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<td>Pig</td>
<td>yes</td>
<td>7 days</td>
<td>p3</td>
<td>T.E. =</td>
<td>=</td>
<td>↑*</td>
</tr>
</tbody>
</table>

Route: I.M.=intramyocardial, I.V.=intravenous, I.C.=intra-coronary, T.E.=trans-endocardial, LVEF= left ventricle ejection fraction, NS= non significant, ?=unknown/not investigated, ==no difference, * p<0.05, ** p<0.01, *** p<0.001. In studies that investigated different groups of ASCs also with additives only groups without additives have been included in this table.
matrix molecules fibronectin and laminin, which are upregulated in the infarcted area after AMI (fibronectin from 12 hours on and laminin from 3 days on). This indicates that in case ASCs are transplanted subsequent to the acute inflammatory period, thus when fibronectin and laminin depositions are high, these proteins might stimulate ASC attachment and differentiation.[43,44]

As depicted in table 1 infarct size reduction in rats indeed was more often non-significant after early ASC injection (within 24 hours), compared with injection after the acute inflammatory period (1 week). We have now shown that application of stem cells 7 days post AMI, leads to a significant reduction in scar size, which was not found when cells were injected at day 1 post AMI.[52] To the best of our knowledge, no other studies have compared the effect of injection of ASCs at different timepoints within one study. Taken together, after the acute inflammation period after AMI (>3 days), the environment in the heart is more favourable for stem cell transplantation. In future studies we will address whether this inflammatory environment can be modified, to improve stem cell therapy, for example by adding inflammatory inhibitors prior to stem cell therapy.

**Culturing of ASCs before transplantation**

Another factor that might influence the fate of stem cells after AMI is the process of culturing these cells before transplantation, which is usually done to achieve a high number of stem cells. Culturing, however, is time consuming, expensive, increases cell size, and, probably most important, affects the functional characteristics of cells.[33,35,52,68] As such it has been shown that already during early culture ASCs loose stem cell markers like CD34.[69] Additionally, we have now found that ASCs express the multi drug resistance protein BCRP, that protects ASCs against ischemia. Also the expression of this protective factor decreases during culture. These data suggest that using the uncultured stromal vascular fraction cells (SVF), which contains ASCs, is more favourable for clinical practice than using long cultured ASCs. This is possible since high numbers of SVF-cells/ASCs can be harvested from a relatively small amount of adipose tissue.[33,35]

The question then arises whether there is a difference in therapeutic effect between uncultured SVF-cells and cultured ASCs. We therefore compared the potential of uncultured SVF-cells and passage 3 ASCs in a rat model of AMI with reperfusion. We have found that uncultured SVF cells reduced infarct size and improved cardiac outcome comparable to cultured ASCs. However, injection with cultured ASCs (p3) occasionally resulted in severe pulmonary complications (thrombo-embolism), which was not found with SVF cells. Since the results in cardiac outcome between uncultured SVF cells and cultured ASCs were comparable, the use of uncultured SVF cells theoretically would be clinically more favourable.

**Administration route**

The route of delivery of stem cells is another important determinant for the fate of the stem cells after AMI. Commonly used administration routes are intra-myocardial (IM) injection (directly, trans-coronary or trans-endocardial) and intravascular delivery (intravenously, or intra-coronary). Several studies have compared the outcome for these different administration routes. Generally it was found that independent of the administration route the majority of the cells is lost, but slightly more cells can be
retraced in the heart after IM injection, compared with intravascular delivery.[70-73] However, IM injections have the disadvantage that they can induce severe complications like for instance wall rupture, arrhythmia and calcifications at the injection site.[74-77]

In the clinical setting intra-coronary injection is usually performed during percutaneous coronary intervention.[74] This method, however, is not optimal since the time point of the percutaneous coronary intervention usually is just before or during the acute inflammation phase of AMI (see above). Other disadvantages of intra-coronary injection are the risk of in-stent restenosis and embolisation of microvessels.[74,75] In contrast, intravenous injection has the advantage that it can be applied at any timepoint desired after AMI. It is even possible to inject stem cells several times intravenously, instead of the application of one single bolus. However, a major disadvantage of intravenous stem cell therapy is the low number of stem cells that reaches the infarcted area, as the majority of the injected stem cells are found to be trapped in the lungs.[78-80] This so called pulmonary first pass effect is at least partly related to the size of the cells injected as stated above.[79] Uncultured cells are smaller compared to cultured ASCs, as culturing of ASCs increases cell size. Therefore, complications after intravenous injection might be reduced by using uncultured SVF cells, as we have also demonstrated.[52]

Figure 2. Factors that we have shown to influence the fate of ASCs after transplantation for AMI.

Faith of ASCs is influenced by the environment in the heart, and the time point of transplantation. Also the route of transplantation and culture of ASCs influences stem cell therapy. + improves stem cell therapy, - reduces chance of successful ASC therapy
In future studies we will further optimize the intravenous delivery method of ASCs/SVF cells, by specifically targeting the cells to the infarction area using microbubbles. Microbubbles are gas-filled bubbles with a diameter of 2-5µm, that originally were used as ultrasound contrast agents.[81] These microbubbles will be labelled with an antibody against ASCs (within the SVF fraction) as well as against a specific target in the infarction area. As such we not only intend to increase the retention time of ASCs within the infarcted area, but also intend to facilitate the binding of this complex to the endothelium by pushing the ASC-microbubble complexes to the vessel wall using the acoustic radiation force of ultrasound.

**Mechanisms through which ASCs can improve cardiac outcome**

Although many studies now have shown that ASCs form a promising new therapy in myocardial infarction, the mechanisms through which ASCs improve cardiac outcome after AMI are not fully elucidated. Studies have postulated different mechanisms for this cardiac improvement, mainly via an effect of ASCs on differentiation or via paracrine effects (figure 3). *In vitro* and *in vivo* studies have shown differentiation of ASCs towards cardiomyocytes and endothelial cells, suggesting that ASCs could improve cardiac outcome by differentiation into these cells.[43,52,63,65,82-88] However, the number of differentiated endothelial cells and cardiomyocytes that could be retrieved in the heart in animal studies was relatively low, suggesting that the improvement of cardiac outcome was not only related to differentiation effects of ASCs.[45,50,52,63] In line with this, several *in vivo* studies have now shown that after treatment with ASCs the number of vessels in the peri-infarction area was increased, albeit without evidence of differentiation of ASCs into endothelial cells.[45,50,52,55]

It was therefore suggested that ASCs might also improve cardiac outcome by so called paracrine effects, indicating that factors excreted by stem cells affect the surrounding cells. Growth factors, cytokines and signalling molecules produced by the infused stem cells can improve vascularisation, reduce inflammation, and favour the viability of cardiomyocytes by inhibition of apoptosis.[89] As such, it has been shown that ASCs secreted significant amounts of vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF)-1, hepatic growth factor and transforming growth factor-β (TGF-β).[90,91] Both the secreted VEGF and IGF-1 have an anti-apoptotic effect on cardiomyocytes subjected to hypoxia *in vitro,*[91] while VEGF also promotes endothelial cell growth and reduced endothelial apoptosis *in vitro.*[90] Finally, it has been suggested that ASCs might also improve cardiac outcome by reducing inflammation or attract resident stem cells to the infarcted area, but this has not been confirmed yet.[92] Also, we did not find a significant effect of ASC therapy on the number of macrophages in the heart after AMI.[52] Taken together, ASCs can improve cardiac outcome after AMI through both induction of differentiation into cardiomyocytes and endothelial cells and through paracrine effects (figure 3).
Figure 3. Mechanisms through which ASCs can improve outcome after AMI. ASCs can directly improve cardiac outcome after AMI through differentiation into cardiomyocytes or endothelial cells. In addition, ASCs secrete factors that promote survival of ischaemic cardiomyocytes and that induce angiogenesis, thereby improving perfusion around the ischaemic area. In addition, it has also been suggested that ASCs might improve clinical outcome after AMI in a paracrine manner by reducing inflammation through modulating protease activity and scar formation and by recruitment of resident cardiac stem cells. HGF= hepatocyte growth factor; IGF= insulin-like growth factor; VEGF= vascular endothelial growth factor.

Conclusions of this thesis

The aim of this thesis was to develop and improve new putative therapies for myocardial infarction. We now have shown that related to the inflammation, the first three days post AMI play a major role in the induction of additional damage to cardiomyocytes, next to ischaemia itself. Specific inflammatory inhibitors, clusterin and PX-18, indeed significantly reduced infarct size, without impairing normal cardiac wound healing, when applied during these first three days post AMI. Therefore these inflammation inhibitors form a promising new therapy in patients with AMI, especially since they not only inhibit inflammatory mediators, but also reduce the pro-inflammatory state of the cardiomyocyte membrane.

To replace lost cardiomyocytes post AMI human ASCs in the SVF fraction of adipose tissue form a potent therapy. These cells are abundantly available and show a high potential of endothelial and cardiomyocyte differentiation. We have now shown that uncultured SVF cells are more favourable over cultured ASCs for therapy after AMI. Furthermore, our studies indicate that SVF-cells/ASCs should be transplanted after the acute inflammation period (>3 days), as the acute inflammatory environment after infarction is a harmful environment also for stem cells. Finally, we have shown that intravenous injection is a safe transplantation route for stem cells that can be used at any desired timepoint post AMI. In conclusion, inflammatory inhibitors and ASCs or their combination, can further improve clinical outcome after AMI, by preventing development of heart failure later on.
References

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