1. Introduction: the acute myocardial infarction

Manifestations of coronary artery disease, such as acute myocardial infarction or chronic ischaemic cardiomyopathy, are caused by a narrowing or obstruction of one or more coronary arteries. Acute myocardial infarction, most frequently caused by occlusion of the coronary artery, induces myocyte necrosis and apoptosis, formation of a fibrotic scar, and ventricular remodelling, including compensatory hypertrophy of the non-ischaemic regions. In contrast, chronic ischaemic cardiomyopathy with left ventricular impairment may be a result of chronic malperfusion of the myocardium. This entity results in hibernating myocardium, which ceases to contribute to the pump function of the heart, but remains viable over a prolonged period of time and may be recruited to pump function again if perfusion is re-established. The current treatment of choice for acute or chronic coronary stenosis/occlusion is revascularization via percutaneous transluminal coronary angioplasty or bypass surgery, which results in a significant improvement in cardiac-related mortality.1–3

Even after successful reperfusion, an additional dysfunction or loss of otherwise vital cardiomyocytes may occur in the ischaemic area, called reperfusion injury.4 This phenomenon may counteract the benefit of myocardial reperfusion.5 In the vascular compartment, reperfusion may induce the so-called ‘no-reflow phenomenon’.7,8 Disruption of blood flow in the microcirculation is caused by adhesion of platelets and neutrophils to the activated endothelium and the swelling of endothelial cells and myocytes.8,9 A common denominator of the various levels of cellular dysfunction is the formation and release of reactive oxygen species (ROS) from myocytes, endothelial cells, and inflammatory cells. Furthermore, during reperfusion ROS are released and lead to leucocyte influx in the ischaemic myocardium. ROS formation and ionic imbalance may subsequently lead to mitochondrial permeability transition pore opening, followed by breakdown of the membrane potential, organelle swelling, and rupture. Although the threshold is not entirely clear, it appears that a critical mass of ruptured mitochondria irreversibly induces complete loss of a cell and necrotic death.10 Studies in animal models of acute myocardial infarction suggest that lethal reperfusion injury accounts for up to 50% of the final size of a myocardial infarct.5 These experimental modulations of reperfusion injury have opened a large field of strategies to improve clinical outcomes in acute myocardial infarction and reduce the risk of heart failure after myocardial infarction. As the therapeutic approaches vary from drug, protein, and peptide therapy to cell and gene therapy, specific application routes may prove appropriate for individual strategies used for these approaches. In this review, we discuss the following issues: (i) current approaches to treatment of acute ischaemia/reperfusion injury; (ii) application techniques for late post-myocardial infarction therapies; and (iii) novel application techniques and their possible clinical applications.

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2. Early ischaemia/reperfusion injury therapy

Aside from the choice of appropriate therapeutic agent for the treatment of ischaemia/reperfusion injury, and the proper therapeutic window, the appropriate treatment site is of utter importance. There are basically two types of applications: systemic and regional delivery. Regional administration comprises anterograde and retrograde administration, as well as direct intramyocardial injection. Further novel methods, such as intrapericardial application, ultrasound-targeted administration, and scaffold-based cell delivery, have recently been introduced. For the treatment of reperfusion injury, the time from onset of ischaemia to revascularization, as well as immediate treatment at onset of reperfusion, is of utmost importance. Therefore, optimal treatment options seem to be systemic, regional (anterograde and retrograde), and as a rather new therapeutic the post-conditioning.

2.1 Systemic application

Systemic intravenous administration is the most common route of drug administration, owing to the simplicity of the approach. Importantly, as acute myocardial infarction is characterized by a reduced perfusion into the target myocardium due to coronary stenosis or thrombi, the efficacy is reduced, and increased amounts of the therapeutic agent are required in order to reach suprathreshold concentrations in the target region. There is also a higher risk of side-effects due to the systemic distribution.11–13 In the experimental setting, the choice of delivery strongly depends on the animal model, because for mice (and rats) the amount of therapeutic agent is rather low, and access to local delivery is difficult. Here, systemic delivery (intravenous or intraperitoneal) is very common.14–17

In large-animal models, such as pigs, systemic administration lacks efficacy compared with local delivery. For example, in a previous study of combined treatment with glutathione (GSH) and cariporide, a sodium/proton exchange inhibitor (NHDE inhibitor), there was failure to improve myocardial function and reduce infarct size when the drugs were applied systemically.12 In contrast, regional application of GSH and cariporide was capable of significantly enhancing myocardial function and reducing infarct size.12 Moreover, systemic administration of embryonic endothelial progenitor cells at the onset of reperfusion failed to improve ischaemia/reperfusion injury. In contrast, local administration of the cells at the same time point and concentration was capable of reducing ischaemia/reperfusion injury in pigs.13

One exception might be gene therapy with a viral vector, which has a tropism for specific tissue e.g. adeno-associated viruses serotype 9 for myocytes.18,19

Up to now, systemic delivery of reperfusion therapy has rarely been used in clinical trials, owing to the low efficacy and the high risk of side-effects. In the REVEAL trial, Najjar and co-workers applied erythropoietin as a single intravenous dose within 4 h of reperfusion. The investigators concluded that a single intravenous bolus of epoetin alpha did not reduce infarct size (Table 1).20 On the contrary, the investigators of the J-WIND trial could show a reduction of infarct size (area under the curve of creatine kinase, acute phase) and improvement of ventricular function (ejection fraction, chronic phase) after systemic intravenous treatment with 0.025 µg/kg and minute atrial natriuretic peptide for 3 days.11 (Table 1). After treatment with atrial natriuretic peptide, 29 of 255 patients had severe hypotension during the acute treatment phase, compared with only one patient

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Table 1 Summary of clinical trials for reperfusion therapy

<table>
<thead>
<tr>
<th>Study</th>
<th>Application route</th>
<th>Therapeutics</th>
<th>Number of patients</th>
<th>Outcome</th>
<th>Study protocol</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>REVEAL20</td>
<td>Intravenous</td>
<td>Epoetin alpha</td>
<td>222</td>
<td>Negative</td>
<td>Randomized, single-blind multicentre</td>
<td>Reduced creatine kinase (area under curve), increased ejection fraction</td>
</tr>
<tr>
<td>J-WIND21</td>
<td>Intravenous</td>
<td>Atrial natriuretic peptide or nicorandil</td>
<td>1216</td>
<td>Positive</td>
<td>Randomized, single-blind multicentre</td>
<td>Improved ventricular function</td>
</tr>
<tr>
<td>AMISTAD II23</td>
<td>Intravenous</td>
<td>Adenosine</td>
<td>2118</td>
<td>Positive</td>
<td>Randomized</td>
<td>Better clinical outcome</td>
</tr>
<tr>
<td>Marzilli et al.28</td>
<td>Intracardiac</td>
<td>Adenosine</td>
<td>44</td>
<td>Positive</td>
<td>Randomized</td>
<td></td>
</tr>
<tr>
<td>Amit et al.30</td>
<td>Intracardiac</td>
<td>Nitroprusside</td>
<td>98</td>
<td>Positive</td>
<td>Randomized, double-blind</td>
<td></td>
</tr>
<tr>
<td>Myoprotect I39</td>
<td>Retrograde</td>
<td>Arterial blood</td>
<td>44</td>
<td>Positive</td>
<td>Randomized</td>
<td>Reduction of infarct size and creatine kinase</td>
</tr>
<tr>
<td>Staat et al.51</td>
<td>Mechanical</td>
<td>Ischaemic post-conditioning</td>
<td>30</td>
<td>Positive</td>
<td>Randomized</td>
<td>Reduction of infarct size and creatine kinase (area under curve)</td>
</tr>
<tr>
<td>Yang et al.52</td>
<td>Mechanical</td>
<td>Ischaemic post-conditioning</td>
<td>41</td>
<td>Positive</td>
<td>Randomized</td>
<td>Reduced apoptosis</td>
</tr>
<tr>
<td>Zhao et al.53</td>
<td>Mechanical</td>
<td>Ischaemic post-conditioning</td>
<td>74</td>
<td>Positive</td>
<td>Randomized</td>
<td>Increased myocardial salvage</td>
</tr>
<tr>
<td>Batker et al.57</td>
<td>Mechanical</td>
<td>Remote ischaemic perconditioning</td>
<td>333</td>
<td>Positive</td>
<td>Randomized</td>
<td></td>
</tr>
<tr>
<td>Piot et al.58</td>
<td>Intravenous</td>
<td>Ciclosporin</td>
<td>58</td>
<td>Positive</td>
<td>Randomized</td>
<td>Reduction of infarct size (MRI and creatine kinase area under curve)</td>
</tr>
</tbody>
</table>
in the control group.\textsuperscript{21} Furthermore, the investigators of the AMISTAD trials could show that intravenous adenosine application after revascularization [thrombolysis or percutaneous coronary intervention (PCI)] reduced infarct size, even though clinical outcome did not improve in the larger trial.\textsuperscript{22,23}

In conclusion, systemic administration is feasible for drugs with a low risk of side-effects, if the therapeutic agent has a tropism to cardiac tissue, or for applications where large-scale production is cost effective and without relevant side-effects and has the advantage of timely treatment without loss of time for revascularization therapy.

2.2 Transvascular applications

2.2.1 Anterograde application

Intracoronary injection, useful for cells, peptides and genes, has been the method of choice in the majority of patient studies with acute myocardial infarction treatment, especially for cell therapy. However, the efficacy of anterograde administration is limited by a variety of factors, such as the endothelial barrier of the coronary artery, or a relatively short transit time (within seconds),\textsuperscript{24–26} combined with an often impaired perfusion in coronary artery disease patients. Accordingly, Emani and co-workers could demonstrate that efficient and reproducible intracoronary gene delivery is dependent upon the infusion flow rate, which in larger animals requires an intraluminal seal (Figure 1A). Excessive flow rate is associated with greater myocardial injury and thereby worsening of the ischaemia/reperfusion injury.\textsuperscript{27} Marzilli and co-workers demonstrated that intracoronary adenosine application (4 mg) after PCI improved ventricular function and prevented the no-reflow phenomena.\textsuperscript{28} In contrast, the study of Desmet et al. did not achieve an increase myocardial salvage [via magnetic resonance imaging (MRI)] after intracoronary adenosine application.\textsuperscript{29} Furthermore, the investigators found no difference in coronary flow and myocardial tissue perfusion in the nitroprusside-treated patients compared with the placebo group.\textsuperscript{30} Even though this study displayed a cardioprotective effect
of pharmacological reperfusion therapy after anterograde delivery, the results have to be confirmed in larger multicentre, randomized trials, because in translation to larger clinical trials most of the therapeutic approaches failed to show protective effects.

2.2.2 Retrograde application

The retrograde coronary vein delivery method provides access to the ischaemic myocardium for target region treatment, even before the onset of reperfusion, if necessary. An advantage of coronary venous application is a slower infusion time in comparison to anterograde delivery (during flow conditions). In addition, the impairment of the anterograde coronary route by coronary artery disease does not affect the coronary veins. We and others could demonstrate that regional administration via selective pressure-regulated retroinfusion was superior to direct intramyocardial injection. Using the retrograde approach, superoxide dismutase infusion immediately before reperfusion was superior to systemic intravenous administration. Regional delivery of superoxide dismutase significantly reduced infarct size and inflammation, and increased myocardial function. In addition, the retrograde application of an intercellular adhesion molecule 1 and a P-selectin antibody, applied immediately before reperfusion, was efficient in limiting ischaemia/reperfusion injury in rats.

Moreover, researchers in our laboratory have demonstrated that ECG-triggered pressure-regulated retroinfusion with suction prevents the myocardium from haemorrhagic incidents and simultaneously reduces systemic distribution, thereby reducing the risk of side-effects. In a pig model of acute myocardial infarction, the selective pressure-regulated retroinfusion of drugs (GSH and cariporide), small peptides (Thymosin β4), and liposomal complementary DNA (cDNA) transfection [vascular endothelial growth factor (VEGF), endothelial nitric oxide synthase (eNOS)], or cells was sufficient to improve myocardial function significantly. In the Myoprotect I study, safety and feasibility of the selective pressure-regulated retroinfusion approach in patients was demonstrated. One limitation of the retrograde approach for the administration of reperfusion therapy is the prolongation of intervention time (5–10 min for selective catheterization of the cardiac veins) and venous valves hindering the access to the target vein.

In summary, retrograde intravenous application is a clinically applicable method; it is effective for pharmacological agents, enabling their homogeneous distribution in the target myocardial area. Coronary artery disease, the disease triggering the acute myocardial infarction, by limiting the access of blood as well as therapeutic agents to the target region, does not affect the venous vascular bed; therefore, drug uptake might benefit from the retrograde approach.

2.3 Post-conditioning

The concept of preconditioning was first described by Murry et al. in 1986, where the authors could show that brief periods of ischaemia followed by reperfusion, before onset of complete ischaemia, could reduce the infarct size after reperfusion. These findings could be confirmed in various experimental settings and showed an involvement of the reperfusion injury salvage kinase (RISK) pathway, as well as mitochondria. This concept of myocardial conditioning was translated to the post-infarct situation by Zhao and co-workers in 2003. Here the investigators reported that short periods of ischaemia and reperfusion after an acute myocardial infarction in dogs resulted in a reduced ischaemia/reperfusion injury. Since this first description of cardioprotective effects of ischaemic post-conditioning, numerous experimental studies on a variety of species (such as mice, rats, rabbits, dogs, and pigs) were successfully performed. Still this rather simple approach of reperfusion therapy, which can easily be performed in the clinical setting, has some limitations. In experimental studies, ischaemic post-conditioning failed to reduce infarct size, when index ischaemia was either too short or too long. Furthermore, the time span between reopening of the coronary artery and the first reocclusion should be rather short to achieve cardioprotection. Based on these promising experimental data, clinical trials for ischaemic post-conditioning were initiated (Table 1). The first clinical trial was performed by Staats and co-investigators. Here 30 patients were randomized to post-conditioning vs. control and underwent four cycles of 1 min ischaemia followed by 1 min of reperfusion directly after stent implantation. Analysis of creatine kinase showed a significant reduction of the area under the curve after post-conditioning treatment. Yang et al. treated 41 patients in randomized study with ischaemic post-conditioning compared vs. control. Post-conditioning was performed immediately after reopening of the coronary artery by three cycles of occlusion and reperfusion, each for 30 s, and then the stent was implanted. They found a clear reduction of creatine kinase release and infarct size at day 7 (in relation to left ventricle) in the post-conditioning group compared with controls.

In a recently published study, Zhao and co-workers compared two different post-conditioning protocols vs. control and analysed the plasma concentrations of Fas. They concluded that three cycles of 60 s inflation and deflation were superior to the protocol with 30 s intervals. However, Freixa and co-workers did not demonstrate a reduction in infarct size after 1 week and 6 months in patients undergoing post-conditioning (four cycles of 60 s inflation and deflation) after PCI post-myocardial infarction. An interesting new approach for ischaemic conditioning is remote ischaemic conditioning. In experimental studies, the cardioprotective effect of preconditioning was shown. Based on these results, a proof-of-concept study was initiated by Bøtker and co-investigators, where 333 patients randomly received a remote ischaemic conditioning via four cycles of blood pressure cuff occlusion and deflation. Interestingly, they found a significantly increased myocardial salvage at day 30 after conditioning treatment. A pharmacological intervention mimicking post-conditioning consists of inhibition of the opening of the mitochondrial permeability transition pore by ciclosporin A. Therefore, Piot et al. conducted a randomized trial, including 58 patients receiving intravenous injection of either 2.5 mg/kg body weight ciclosporin A or saline directly before PCI. Analysis of this pilot trial displayed a significant reduction of infarct size on MRI at day 5. Taking these results together, post-conditioning is a promising new technique for reperfusion therapy, which can easily be transferred to the clinical situation, but for the assessment of the cardioprotective effect for patients with acute myocardial infarction, larger clinical trials need to be performed to confirm these data.

3. Late post-myocardial infarction therapies

Even after successful revascularization, a high number of patients (30%) will develop chronic heart failure. Improvement of reperfusion therapy, as described above, is capable of reducing the reperfusion part of the infarct size, whereas due to ischaemia these...
patients still will develop a myocardial scar depending on the site of occlusion and duration of ischaemia. Therefore, besides the treatment of reperfusion injury, application of late post-myocardial infarction therapies might useful. Targets of late post-myocardial therapies are angiogenesis, arteriogenesis, and contractility, which might be achieved through gene or cell therapy, and require different delivery approaches as the acute therapy.

### 3.1 Anterograde application

To overcome some of the limitations of intracoronary application for gene delivery, in pre-clinical trials transgene administration was combined with agents that increase permeability of the vascular bed, such as VEGF, substance P, or histamine, thereby allowing a higher myocardial transfection efficacy. However, side-effects, such as hypotension and increased permeability, may limit the clinical use of these agents.

For cell therapy, occlusive as well as non-occlusive anterograde delivery is performed. In case of the non-occlusive administration mode, a small catheter or a non-inflated over-the-wire balloon is placed selectively in the target vessel, and cell injection is performed via continuous infusion or repetitive slow hand injection. In most clinical studies, the stop-flow method is used by inflating the over-the-wire balloon mildly, preventing spillover and rapid washout of stem/progenitor cells after ischaemia/reperfusion (Figure 1A).

In the REPAIR-AMI as well as in the TOPCARE-AMI study, endothelial progenitor cells were applied to the infarct-related artery (at the site of stent implantation) via an over-the-wire balloon during stop-flow conditions (Table 2). Therefore, the balloon was occluded at a low pressure, to block the anterograde blood flow completely for 3 min. During blockade of the blood flow, 3.3 mL of the cell suspension with progenitor cells was applied to the coronary artery distal to the balloon occlusion, thereby allowing for cell adhesion and potential cell transmigration in the target region. This intervention was repeated three times, with 3 min of reperfusion, to apply the final volume of 10 mL cell suspension to the ischaemic myocardium. In the BOOST trial, Wollert and colleagues followed a similar application protocol of stop-flow injection. The entire bone marrow cell preparation was infused during four to five coronary occlusions, each lasting 2.5–4 min. Between occlusions, the coronary artery was reperfused for 3 min. In the IACT study, four to six fractional infusions were performed with an angioplasty balloon catheter during stop-flow conditions. Again, cells were infused directly into the infarct-related artery over 2–4 min with a volume of 3–5 mL, allowing for recovery between the fractional infusions.

In the LateTIME trial, delivery of bone marrow cells was performed via intracoronary infusion of the cells in patients with a reduced left ventricular function 2–3 weeks after acute myocardial infarction (AMI) and PCI (Table 2).

Roger Hajjar and his group developed a specific catheter-based anterograde intracoronary delivery with coronary venous blockade for viral gene delivery (Figure 1B). In brief, a wedge balloon was applied to the great cardiac vein or the anterior intraventricular vein and inflated until coronary venous occlusion was confirmed by angiography. Thereafter, an over-the-wire balloon was advanced to the left anterior descending artery distal to the first diagonal branch or distal to the first marginal branch. After occlusion of the artery was confirmed by angiography, local gene delivery was performed over 3 min after intracoronary adenosine injection. Even though a single anterograde application was sufficient to transduce the target myocardium, the occlusion of the coronary vein significantly enhanced reporter gene activity in the target region (Table 2).

**Table 2** Summary of clinical trials for late post-myocardial infarction therapies

<table>
<thead>
<tr>
<th>Study</th>
<th>Application route</th>
<th>Therapeutics</th>
<th>Number of patients</th>
<th>Outcome</th>
<th>Study protocol</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>REPAIR-AMI58</td>
<td>Anterograde</td>
<td>Endothelial progenitor cells</td>
<td>204</td>
<td>Positive</td>
<td>Randomized, multicentre</td>
<td>Improved left ventricular contractility</td>
</tr>
<tr>
<td>TOPCARE-AMI66</td>
<td>Anterograde</td>
<td>Endothelial progenitor cells</td>
<td>20</td>
<td>Positive</td>
<td>Non-randomized for control group</td>
<td>Improved left ventricular contractility</td>
</tr>
<tr>
<td>BOOST69</td>
<td>Anterograde</td>
<td>Bone marrow cells</td>
<td>60</td>
<td>Positive</td>
<td>Randomized</td>
<td>Improved left ventricular function</td>
</tr>
<tr>
<td>LateTIME71</td>
<td>Anterograde</td>
<td>Bone marrow cells</td>
<td>89</td>
<td>Negative</td>
<td>Randomized, double-blind</td>
<td>Decreased cardiovascular events</td>
</tr>
<tr>
<td>CUPID73</td>
<td>Anterograde with retrograde block</td>
<td>AAV1/SERCA2a</td>
<td>39</td>
<td>Positive</td>
<td>Randomized, double-blind multicentre</td>
<td></td>
</tr>
<tr>
<td>TABMMI study77</td>
<td>Intramyocardial</td>
<td>Bone marrow cells</td>
<td>10</td>
<td>Positive</td>
<td>Non-randomized single-centre</td>
<td>Increased ejection fraction</td>
</tr>
<tr>
<td>NORHTERN79</td>
<td>Intramyocardial</td>
<td>VEGF cDNA</td>
<td>93</td>
<td>Negative</td>
<td>Randomized, double-blind multicentre</td>
<td>Improved regional wall motion</td>
</tr>
<tr>
<td>Euroinject One80</td>
<td>Intramyocardial</td>
<td>VEGF cDNA</td>
<td>80</td>
<td>Positive</td>
<td>Randomized, multicentre</td>
<td>Reduction of infarct size, improved ejection fraction</td>
</tr>
<tr>
<td>MYSTAR82</td>
<td>Intramyocardial</td>
<td>Bone marrow cells</td>
<td>60</td>
<td>Positive</td>
<td>Randomized</td>
<td>Positive for angina frequency and exercise time</td>
</tr>
<tr>
<td>Losordo et al.83</td>
<td>Intramyocardial</td>
<td>GCSF+ CD34+ cells</td>
<td>164</td>
<td>Positive</td>
<td>Randomized, double-blind multicentre</td>
<td>Negative</td>
</tr>
<tr>
<td>MAGIC91</td>
<td>Intramyocardial</td>
<td>Myoblast</td>
<td>120</td>
<td>Negative</td>
<td>Randomized, double-blind multicentre</td>
<td>Negative</td>
</tr>
</tbody>
</table>
Kaye et al. established an alternative delivery method. An occlusive balloon recovery catheter was placed in the coronary vein and connected to a suction device containing a roller pump and an oxygenator membrane. Oxygenated perfusate was then infused into the target coronary artery via a non-occlusive catheter. Recirculation was performed for 10 min, while the pump flow was set such that sinus pressure did not exceed 5 mmHg. The authors could show that expression of a reporter gene was higher in the group with recirculation than with intracoronary delivery, and systemic contamination with the vector was diminished. Recalculating adeno-associated virus type 1/sarcoplasmic reticulum Ca2+ ATPase (AAV1/SERCA2a) was capable of improving myocardial function compared with intracoronary infusion (Table 2).

Even though anterograde administration combined with venous blockade has primarily been used for gene therapy in ischaemic heart disease, it seems feasible for plasmid, peptide and cell therapy. The approach reduces systemic contamination and can either prolong contact time or enhance infusion pressure and thereby increase the concentration of therapies in the target myocardium.

### 3.2 Retrograde application

Retroinfusion via the coronary venous system has gained increasing interest for gene and cell therapy, because transduction within the myocardium is more homogeneous than anterograde or direct intramyocardial administration. In addition, retrograde coronary venous application leads to slower transition times of the infused agent and might thereby increase adhesion time (of viral vectors as well as of cells) and thereby enhance efficacy. Patients with coronary heart disease often have impaired endothelial function and stenosis in the primary infarcted vessel, as well as in the other coronary arteries; therefore, anterograde administration might not be the preferable application route. Combining the retrograde application via ECG-triggered retroinfusion with a suction device significantly reduces the systemic contamination, which might be even more important for pro-angiogenic therapy than for the side-effects of reperfusion therapies.

In pr-clinical animal models (pigs) of chronic myocardial ischaemia, local delivery of pro-angiogenic therapies ([cDNA-endothelial nitric oxide synthase, adenosine encoding hypoxia-inducible factor-1α, and adeno-associated viral vectors encoding human VEGF and human platelet-derived growth factor (PDGF)]) was sufficient not only to induce angiogenesis, but also to improve perfusion, as well as myocardial function. In summary, selective pressure-regulated retroinfusion seems to be a favourable approach for gene therapy (and potentially for cell therapy), because a homogeneous transduction in combination with reduced systemic contamination is achievable.

### 3.3 Intramyocardial applications

Although vascular access is advantageous for a variety of therapeutic formulations, direct intramyocardial application may be sought either if the therapeutic agent should remain strictly within the myocardial compartment to avoid serious side-effects or if it lacks the machinery to travel across the vascular wall, such as cardiomyocyte progenitor cells. This regional administration of therapeutic agents to the myocardium may be performed via transluminal endocardial injection or after thoracotomy into the epicardium.

#### 3.3.1 Transendocardial intramyocardial application

For transluminal endocardial access, a variety of injection catheters are available. The main difference between the injection catheters is the visualization of the injection sites. Fluoroscopic two-dimensional catheters, such as the BioCardia Helical Infusion catheter (BioCardia, South San Francisco, CA, USA) and Stilettos (Boston Scientific, Natick, MA, USA), and three-dimensional guidance systems, such as the Myostar Injection Catheter (BioSense Webster, Diamond Bar, CA, USA), in combination with NOGA (catheter based cardiac electromechanical mapping), allow for detailed targeting of the injection site.

The Helical infusion catheter catheter (BioCardia) is manoeuvred by a Morph Deflectable Guide Catheter into the left ventricle (Figure 2A). By virtue of the screw head, the Helical catheter is fixed at the targeted entry site of the myocardium during injection (Figure 2A). In pigs, the administration of radioactively labelled albumin showed a high concentration of radiolabel at the treatment site. The catheter was also used for transendocardial autologous bone marrow cell transplantation in a non-randomized single-centre pilot study (TABMMI study) (Table 2). Bone marrow cells were implanted in 7 ± 3 injection sites in the infarct and peri-infarct zone, defined by orthogonal angiography and echocardiography. No procedural complications or sustained arrhythmias were observed; therefore, the investigators concluded that the helical needle injection is safe. Pre-clinical and clinical studies are currently underway using the BioCardia Helical Infusion System to deliver genes and cells to treat heart failure, myocardial infarction, and ischaemia.

In an alternative approach, the NOGA three-dimensional electromagnetic cardiac mapping system ( Biosense Webster) enables reconstruction of the left ventricle in a three-dimensional manner. The unipolar voltage map allows the investigator to discriminate between normal myocardium, infarct areas, and the border zone of the infarct (Figure 2B). In combination with the Myostar Injection Catheter (BioSense Webster) exact injections into the target region are possible. After feasibility studies in large animals, the NOGA system has already been used in a variety of clinical trials. In the NORTHERN trial and the Euroinject One trial, the NOGA system was used to inject VEGF cDNA into the myocardium. In a next step, Kastrup and co-workers used the NOGA XP system to apply an adenoviral VEGF121 regionally to the ischaemic myocardium (Table 2). They could demonstrate that the intramyocardial injection was safe, but failed to improve patient’s outcome. Furthermore, the NOGA injection system was used to apply bone marrow mononuclear cells to the ischaemic myocardium. In the MYSTAR randomized trial, the cells were applied via direct injection to the infarct border zone at 10 injection sites. This delivery approach was combined with a direct infusion of the cells to the target vessel. The investigators reported a significant improvement in electric and mechanical function, as well as in myocardial perfusion at the intramyocardial injected sites. Losordo and co-workers used the NOGA system in a pilot study to apply autologous CD34+ cells after granulocyte-colony stimulating factor (GCSF)-mediated mobilization. The total dose of cells was divided into 10 intramyocardial transendocardial injections. These intramyocardial injections of cells or saline did not result in cardiac enzyme elevation, perforation, or pericardial effusion. No incidence of ventricular tachycardia or ventricular fibrillation occurred during the intramyocardial injections, even though cell mobilization and collection led to cardiac enzyme elevation. Analysis of exercise tolerance test and anginal episodes revealed a better
3.3.2 Epicardial intramyocardial application

An alternative access to the myocardium is the epicardial direct intramyocardial injection, which requires a thoracotomy. In experimental studies, especially with rodents this delivery method is favoured for gene therapy, either naked DNA or viral gene transfection and cell transplantation. In clinical trials, this intramyocardial injection is combined with a thoracotomy done for bypass surgery or over a mini-thoracotomy. In the MAGIC trial on patients undergoing elective bypass surgery, myoblasts were injected in the akinetic myocardial wall with the use of a 27-gauge needle. Up to 30 injections were performed to apply $400 \times 10^6$ or $800 \times 10^6$ cells into the epicardium. This trial failed to meet the primary end-point. Mocini and colleagues injected autologous bone marrow mononuclear cells in patients undergoing bypass surgery. They could show that the direct injection of bone marrow mononuclear cells in the myocardium
during coronary artery bypass grafting is feasible and safe. Furthermore, the patients showed an improvement in left ventricular ejection fraction and wall motion score index. As there were only 36 patients, larger studies are needed to assess the efficacy of such an approach in patients undergoing coronary artery bypass grafting (92) (Table 2).

Taken together, both administration methods have the advantage of relatively low systemic contamination and similar efficacy. In particular, for autologous myoblast application in pigs,90 and for fibroblast of relatively low systemic contamination and similar efficacy. In particular, for autologous myoblast application in pigs,90 and for fibroblast growth factor 2 delivery using the NOGA system,93 experimental studies indicate that optimized endocardial intramyocardial injection is as effective as the transendocardial delivery.

Disadvantages of these delivery methods are the risk of bleeding, the need for a thoracotomy for the epicardial approach, release of biomarkers after intramyocardial injection, and a relatively low distribution around the injection side (approximately 5 mm for gene therapy).74,94,95 Of note, Laham et al. indicated that optimized endocardial intramyocardial fibroblast growth factor 2 delivery using the NOGA system is as effective as the transendocardial delivery.93

4. Novel techniques

4.1 Ultrasonic gene and drug delivery

Ultrasound-targeted microbubble destruction has evolved as a promising tool for organ-specific gene and drug delivery. Since generation ultrasound contrast agents were developed for myocardial contrast echocardiography,96 gas-filled microbubbles with improved stability (up to several minutes) have become available. This new therapeutic approach could combine the following two major issues of drug/biological delivery: (i) low invasiveness; and (ii) high organ specificity. Remarkably, loading microbubbles with plasmid cDNA or viral vectors followed by ultrasound destruction in the target region increased transfection rate.97–99 Furthermore, high-amplitude oscillations of microbubbles increase capillary and cell membrane permeability, thus facilitating tissue and cell penetration of the released agents.100 In vivo, the ultrasound-targeted microbubble gene transfer (cDNA and viral vectors) increased reporter gene expression eight- to 200-fold, not only in mice and rats, but also in dogs and pigs (for review see 101). In rats, the local overexpression of stromal-derived factor-1 via ultrasound microbubbles significantly enhanced progenitor cell recruitment, and thereby increased cardiac function and perfusion.102 Taken together, ultrasound-targeted microbubble destruction may become an important therapeutic tool for treatment of cardiovascular disease, not only for reperfusion injury, but also for anti-inflammatory treatment in atherosclerosis, an inducer of acute myocardial infarction.103,104 Still, most of the studies using therapeutic gene transfer have been performed in small-animal models.105 As a next step towards a clinical application, pre-clinical large-animal studies will reveal the therapeutic potential of ultrasonic gene delivery.

4.2 Epicardial delivery

The pericardium creates an isolated fluid-filled compartment enclosing the whole heart. This seems to be an ideal compartment for local delivery of drugs, cells and vectors to the heart. It has already been shown that nitric oxide donors can be applied successfully to the pericardial space and lead to coronary dilatation.105 Lazarous and co-workers could demonstrate that adenovirus administration in the pericardium was capable of inducing basic fibroblast growth factor expression in dogs.106 Even though the pericardial delivery approach seems to be promising with respect to the concentration of therapeutic agent in the heart and low systemic contamination, the endocardial deposition of therapeutic agents requires pericardial puncture or a surgical approach.107 The development of a minimally invasive delivery system might offer an attractive local delivery approach in the pericardium. Ladage and co-workers developed a method of liquidified gel foam particles associated with biological agents, which were applied into the pericardial space via a subternal puncture under fluoroscopic guidance. This method proved to be successful in delivering mesenchymal stem cells and adenovirus to the infarcted heart.108 For ischaemia/reperfusion injury, this might be a useful approach for drug delivery prior to vessel reopening, thereby targeting the acute phase of reperfusion injury.

4.3 Scaffold-mediated cell delivery: surgical approach

Engraftment, survival and function of mesenchymal stem cells in the ischaemic tissue is still an issue in cell therapy. Therefore, Le Visage et al. designed a polysaccharide-based porous scaffold for delivery of mesenchymal stem cells to the heart; thereby, engraftment as well as survival could be increased after transplantation of mesenchymal stem cells to rat hearts after myocardial infarction.109 In a second study, a biological composite scaffold was established to apply mesenchymal stem cells to the infarcted myocardium. To a decellularized human myocardium, fibrin hydrogel with suspended cells was added. This cell–matrix composite was implanted onto infarcted rat hearts and, in combination with preconditioned mesenchymal stem cells, led to improved left ventricular function and vascularization in the infarcted heart.110 This scaffold approach might increase cell homing and survival and thereby enhance the cardioprotective effect of the cells. However, application of the scaffold to the myocardium is associated with an invasive approach, because direct access to the epicardium is prerequisite. The epicardial delivery of a scaffold (polysaccharide of decellularized myocardium) might lead to local inflammation in larger animals and humans. This approach seems to be more feasible for experimental studies, or for patients undergoing coronary artery bypass operation.

5. Conclusion

The revascularization therapy of an acute myocardial infarction has opened a large field of treatment options for reperfusion injury. As the therapeutic approaches vary from drug, protein, and peptide therapy to cell and gene therapy, specific applications seem to be optimal for the different therapeutic agents. To avoid systemic contamination and side-effects, local application should be the preferred route. Anterograde delivery is feasible for drug and cell therapy, if used during low- or no-flow conditions, because retrograde delivery requires an additional vascular access and some training for catheterization of the coronary veins. For gene therapy (especially of pro-angiogenic agents), the retrograde venous approach is preferable, because the venous endothelium does not suffer from the coronary artery disease. Intramyocardial applications have the advantage of low systemic contamination, but the disadvantages of a high risk of bleeding, ventricular tachycardia, and a limited dissemination of the therapeutic agent (and the epicardial approach requires a thoracotomy). Still this approach seems to be feasible for cell application,
especially with the NOGA system, where the therapeutic agent can be applied in the designated area. Ultrasonic gene and drug delivery, a new approach lacking pre-clinical studies, seems to combine systemic delivery favourably with administration to the target region, mediated through the microbubble destruction, whereas other new techniques (epicardial delivery and scaffold-mediated cell delivery) are still in a developmental stage. In conclusion, the regional delivery of any therapeutic agent should be the preferred approach; the choice of the application route is dependent on agent, treatment goal, and the time window during ischaemia and reperfusion in which the targeted therapy is applied.

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