Electrophysiological effects of intracoronary transplantation of autologous mesenchymal and endothelial progenitor cells

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Aims Autologous stem cell transplantation has been successfully used for repair of infarcted myocardium, but concerns have been raised regarding its pro-arrhythmic potential. This study aimed at using electrophysiological assessment, and the monitoring and data storage capacity of implanted cardioverter defibrillators (ICDs), in order to evaluate the possible proarrhythmic potential of stem cell transplantation.

Methods Five patients with a history of previous anteroseptal myocardial infarction and an implanted ICD for ventricular arrhythmias underwent intracoronary transplantation of autologous bone marrow-derived and culture-expanded mesenchymal stem cells in combination with endothelial progenitors.

Results There was evidence of myocardial repair in three patients in whom segmental left ventricular wall motion improvement was detected on stress echocardiography. Before stem cell transplantation, clinical non-sustained ventricular tachycardia and inducible monomorphic ventricular tachycardia, or ventricular flutter at electrophysiology study were demonstrated in all patients. At 16-36 months follow-up, interrogation of the ICD failed to detect sustained or non-sustained ventricular arrhythmia in any patient. At repeat electrophysiology study, sustained ventricular arrhythmia was induced in two patients.

Conclusion Intracoronary transplantation of autologous mesenchymal and endothelial progenitor cells does not appear to be arrhythmogenic in humans. Further studies are needed on this important clinical issue.

Keywords Mesenchymal stem cells; Endothelial progenitors; Autologous stem cell transplantation

Introduction

Transplantation of skeletal myoblasts has been successfully used for repair of infarcted myocardium in humans but at a risk of inducing life-threatening arrhythmias.1-3 Intracoronary autologous stem cell transplantation has also been shown to improve perfusion and contractility of the infarcted left ventricular area without reported proarrhythmic complications.4-6 However, experimental studies have suggested that stem cell-derived cardiomyocytes may also demonstrate arrhythmic potential.7-10 The relevance of these findings to the human heart is unknown.

We used serial electrophysiological assessment, as well as the benefit of implanted cardioverter defibrillators (ICDs) with extensive monitoring and data storage capacity in five patients who were treated with autologous stem cell transplantation highly enriched with mesenchymal stem cells (MSCs) and retaining the starting population of endothelial progenitors to evaluate the possible proarrhythmic potential of such a treatment.

Methods

Five patients with a history of previous anteroseptal infarct treated with primary angioplasty and stent deployment underwent autologous stem cell transplantation. All patients had an ICD implanted due to clinical non-sustained ventricular tachycardia and inducible ventricular tachycardia or flutter at electrophysiology study. Implanted cardioverter defibrillators implanted were Medtronic (Minneapolis, MN, USA) Gem 7227 (Patient 1), Medtronic GEM III VR 7231 (Patient 2), Medtronic Marquis VR 7230 (Patients 3 and 5), and Guidant (St Paul, MN, USA) Vitality VR 1870 (Patient 4).

These patients participated in the programme of autologous stem cell transplantation in patients with acute and chronic myocardial
infarction that commenced in our departments in February 2003. Previously reported studies have used whole bone marrow mononuclear or neangiogenesis-inducing endothelial progenitor cells (EPCs). In this trial, we used a combination of bone marrow-derived and culture-expanded MSCs with EPCs. Mesenchymal stem cells reside in various niches and are characterized by the potential to differentiate into multiple lineages, such as osteocytes, adipocytes, chondrocytes and muscle cells, as well as the ability to display immunosuppressive potential. Endothelial progenitor cells originate from bone marrow or mobilized peripheral blood, and have the potential to differentiate into endothelial cells, thereby contributing to angiogenesis and vascularization.

The present study was ethically approved by our Institutional Review Board and all patients provided a written informed consent fully describing the experimental nature of the protocol.

**Stem cell preparation**

The day following the coronary angiography or angioplasty, bone marrow aspirates (15–20 mL) were obtained from the iliac crests of patients under local anaesthesia. Bone marrow mononuclear cells were isolated by density gradient centrifugation using Ficoll separating solution (Biochrom AG) and cultured in DMEM-LG (Life Technologies), supplemented with 10% foetal calf serum (FCS, Biochrom AG, lot selected for optimal growth of MSCs). On day 3, non-adherent cells were removed and fresh culture medium was added. On day 7, cultures were tested for sterility, and adherent cells were detached, washed, resuspended in 3 mL saline (B. Braun, Meslungen, AG), and transferred to the operation room. A small quantity of cells was kept for phenotyping by flow cytometry both on day 0 and day 7. Cells stained positive with anti-CD29, -CD44, -CD105, SH2, and SH3 antibodies; and negative for anti-CD34 and -CD45 represented MSCs, whereas cells stained positive with anti-CD31, -CD105, -CD133 and -KDR represented EPCs. All solutions used were of good manufacturing practice and all procedures were performed under good laboratory practice conditions.

Mesenchymal stem cells can be expanded in vast numbers in 1 month of culture. However, this results in loss of EPCs from the culture and thus, a 1-week culture period was selected for our study. In addition, we have developed a culture protocol allowing us to obtain large numbers of MSCs in a relatively short period of time; thus smaller quantities of bone marrow than those used in other studies are required.

**Intracoronary transplantation**

For cell transplantation the left anterior descending coronary artery was catheterized; an over-the-wire balloon was positioned at the stent and inflated at 6 atm for 2 min. During this time a 1.5 mL cell suspension, containing 2.5–4.9 \times 10^6 cells, was infused distally to the occluding balloon through its central lumen. The procedure was repeated once following a 3-min deflation of the balloon for restoration of coronary flow.

**Left ventricular function assessment**

Qualitative evaluation of left ventricular systolic function was based on the division of the left ventricle into 16 segments, according to the model proposed by the American Society of Echocardiography. A segment was characterized hypokinetic or akinetic when endocardial excursion was <5 and <2 mm, respectively. Segmental wall motion was scored as 1, 2, 3, or 4 if it was normal, hypokinetic, akinetic, or dyskinetic, respectively. Stress echocardiography was performed with dobutamine infusion immediately before cell implantation and 4 months later for the assessment of myocardial viability as previously described.

**Electrophysiological study**

For electrophysiological testing (EPS), patients were studied in the post-absorptive state, under sedation with diazepam and dexamethasone, and after beta-blockers or other antiarrhythmic agents had been discontinued for 3 days. No patient was receiving amiodarone. Local anaesthesia was administered, and under fluoroscopic control multipolar electrodes were introduced into the right atrium and the His-bundle area, the coronary sinus, and the right ventricle. Bipolar electrograms were recorded from the distal pair of electrodes, filtered at 30–500 Hz, amplified at gains of 20–80 mm/mV and displayed and acquired on a Bard, LabSystem Duo, together with surface electrocardiograms. Programmed electrical stimulation was accomplished according to the standard Wellens protocol. Initiation of ventricular tachycardia was facilitated by isoprenaline infusion of 2–5 \mu g/min aiming at a target heart rate of 120 bpm. Electrophysiology study was performed immediately before, and at 12 months following stem cell transplantation.

**Results**

**Stem cells transplantation**

The number of cells transplanted and the percentage of each type of progenitors for each patient are summarized in Table 1. All transplantation procedures were uneventful. Patients were discharged on the same medication as before, consisting of beta-blockade, an angiotensin receptor blocker, a statin, aspirin, and clopidogrel as indicated. Left ventricular function parameters, pre- and post-transplantation, are listed in Table 2.

**Ventricular arrhythmia**

All patients had episodes of non-sustained ventricular tachycardia before ICD implantation, and in all patients sustained monomorphic ventricular tachycardia or ventricular flutter was induced at electrophysiological study (Table 3). Implanted cardioverter defibrillators interrogation provided data on arrhythmia episodes during follow-up and, in three patients, the period preceding stem cell transplantation. During this period, ventricular tachycardia was detected in one of three patients. Following stem cell transplantation, ICD interrogation failed to detect any episodes of sustained or non-sustained ventricular arrhythmias in any patient during the follow-up period (Table 4).

At repeat electrophysiological study during follow-up sustained monomorphic ventricular tachycardia or ventricular flutter was induced in two patients. Non-sustained arrhythmia was induced in three patients and in two of them only following isoprenaline infusion.

### Table 1 Numbers and type of stem cells infused

<table>
<thead>
<tr>
<th>Patient</th>
<th>Number of stem cells infused (x10⁶)</th>
<th>MSCs (%)</th>
<th>EPCs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>2.5</td>
<td>51</td>
<td>20</td>
</tr>
<tr>
<td>Patient 2</td>
<td>4.9</td>
<td>57</td>
<td>30</td>
</tr>
<tr>
<td>Patient 3</td>
<td>2.5</td>
<td>64</td>
<td>22</td>
</tr>
<tr>
<td>Patient 4</td>
<td>3.0</td>
<td>70</td>
<td>10</td>
</tr>
<tr>
<td>Patient 5</td>
<td>3.9</td>
<td>72</td>
<td>11</td>
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</table>
Discussion

This study represents part of a novel clinical trial for transplantation of mesenchymal and endothelial stem cells in the human infarcted myocardium.6 Our results indicated that in patients with an anteroseptal myocardial infarction, MSCs, and endothelial progenitor (EPCs) transplantation was an independent predictor of improvement of non-viable tissue at 4 months post-transplantation.6

Safety of MSCs

The use of FCS for the culture of cells to be used in clinical trials raises potential hazards that cannot be neglected. However, calf serum has been used for MSCs culture in several clinical trials for the treatment of various disorders. Infusion of large numbers of MSCs at different sites (such as intravenously, intracoronary, in the middle cerebral artery or in the surgically-exposed spinal cord) has so far not been reported to cause any significant side effects.20 Mesenchymal stem cells are well known not to be inherently immunogenic, as well as to escape recognition by the immune system.21 Moreover, in a study using FCS where medium-derived antigens were internalized by MSCs, the immunogenic doses of MSCs were in the range of 108, while they were cultured in 20% FCS.22 This is far from the numbers of cells used in our study, where furthermore only 10% FCS was used.6 Cumulative data have been recently published indicating a potential immunosuppressive role of MSCs such as prevention of transplant rejection,23 and treatment of acute graft vs. host disease.24 Mesenchymal stem cells have been used for regeneration of various organs and they do not appear to be rejected by the host, even in allogeneic or xenogeneic models.25

Arrhythmogenic potential

Recently, an experimental patch-clamp study on mice myocardial cells has shown that cardiomyocytes derived from embryonic stem cells display heterogeneity of the action potential morphology, slower upstroke velocities, protracted automaticity, prolonged action potentials, and easily inducible triggered arrhythmias.7 Increased sympathetic nerve sprouting, a known substrate for cardiac arrhythmia, has also been detected in swine hearts that received implants consisting of isolated MSCs together with fresh bone marrow mononuclear cells,8 and shortening of ventricular refractoriness has been seen in animals after intravenous administration of mesenchymal cells.9 In a recent experimental study, human MSCs cocultured with neonatal rat ventricular myocytes also produced an arrhythmogenic substrate that facilitated re-entry.10 No human study has addressed this issue so far. The lack of clinical arrhythmia during 16–36 months following transplantation, as well as the reduced inducibility of sustained rhythms at electrophysiological study that was demonstrated by our study is reassuring. Previous experience with skeletal myoblast transplantation has shown that proarrhythmia usually occurs early, within

Table 2  Left ventricular function pre- and post-stem cell transplantation

<table>
<thead>
<tr>
<th></th>
<th>EDV pre</th>
<th>EDV post</th>
<th>ESV pre</th>
<th>ESV post</th>
<th>EF pre (%)</th>
<th>EF post (%)</th>
<th>Akinetic myocardial segments (DES) post-MSCs/EPCs transplantation</th>
<th>Akinetic myocardial segments (DES) pre-MSCs/EPCs transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>138</td>
<td>91</td>
<td>80</td>
<td>54</td>
<td>35</td>
<td>38</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Patient 2</td>
<td>155</td>
<td>147</td>
<td>108</td>
<td>94</td>
<td>34</td>
<td>36</td>
<td>6</td>
<td>5</td>
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<tr>
<td>Patient 3</td>
<td>144</td>
<td>141</td>
<td>78</td>
<td>89</td>
<td>39</td>
<td>37</td>
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<td>5</td>
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<tr>
<td>Patient 4</td>
<td>239</td>
<td>237</td>
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<td>164</td>
<td>30</td>
<td>30</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Patient 5</td>
<td>182</td>
<td>174</td>
<td>110</td>
<td>105</td>
<td>30</td>
<td>35</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

EDV, end-diastolic volume; ESV, end-systolic volume; EF, left ventricular ejection fraction (%); pre, pre-stem cell transplantation; post, post-stem cell transplantation; DES, dobutamine stress echocardiography; MSC/EP, mesenchymal stem cells/endothelial progenitors.

Table 3  Patient characteristics and timing of ICD and stem cell transplantation

<table>
<thead>
<tr>
<th></th>
<th>Sex</th>
<th>Age</th>
<th>Time from AMI to MSCs/EP transplantation (months)</th>
<th>Time from ICD to MSCs/EPCs transplantation (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>Male</td>
<td>42</td>
<td>52</td>
<td>51</td>
</tr>
<tr>
<td>Patient 2</td>
<td>Male</td>
<td>32</td>
<td>24</td>
<td>19</td>
</tr>
<tr>
<td>Patient 3</td>
<td>Male</td>
<td>35</td>
<td>3</td>
<td>_a</td>
</tr>
<tr>
<td>Patient 4</td>
<td>Male</td>
<td>55</td>
<td>96</td>
<td>16</td>
</tr>
<tr>
<td>Patient 5</td>
<td>Male</td>
<td>56</td>
<td>2</td>
<td>_a</td>
</tr>
</tbody>
</table>

As regards karyotyping, we do not perform relevant tests for every patient, but we do periodically examine specimens. However, a 2-week culture period does not result in chromosomal abnormalities of MSCs in our own experience. Chromosomal instability may occur as a result of very extended in vitro expansion, as observed after numerous population doublings (passages 12–17, which, to our knowledge corresponds to >4 months of culture) and only in murine MSCs, since this result failed to be replicated with human MSCs.26
the initial weeks following the procedure.\textsuperscript{1–3} The presence of an ICD provided a unique opportunity to obtain reliable data on the incidence of any arrhythmia in this population. Our study failed to demonstrate ventricular arrhythmias in patients who were prone to arrhythmia due to their condition regardless of stem cell transplantation. Of course, in these reports either epicardial (intra-operative)\textsuperscript{1,3} or endocardial (with the assistance of electromechanical mapping)\textsuperscript{3} injections of myoblasts were undertaken; thus these results may not be comparable with ours that were achieved following intracoronary transplantation.

From the theoretical point of view, however, it seems that myoblasts and stem cells may differ in their inherent electrophysiological properties and in their ability to couple electromechanically with host cardiomyocytes.\textsuperscript{27} Probably due to differences in expression of the proteins N-cadherin and connexin-43\textsuperscript{28,29} as well as interaction with L-type calcium channels,\textsuperscript{30} electromechanical coupling has not yet been demonstrated between skeletal grafts and cardiac muscle cells.\textsuperscript{31–33} One might speculate, therefore, that arrhythmias are more likely to occur after myoblast than after stem cell transplantation.

Study limitations

This was a small and non-randomized series and should be regarded as preliminary experience. Further clinical experience is necessary before reaching valid conclusions regarding the safety of autologous stem cell transplantation in patients with chronic myocardial scars. Still, proarrhythmia with myoblasts has been reported in up to 40\% of patients and a similar incidence should have resulted in arrhythmia episodes in at least two of our patients. Then, tachycardias with a cycle length below the preset detection interval of the ICD might have been undetected. However, no such slow arrhythmias were seen before transplantation and none of the arrhythmias, that actually occurred before cell transplantation, was detected. Furthermore, at electrophysiological study arrhythmias were more difficult to induce than before stem cells transplantation. Finally, the exact mechanism of myocardial remodelling following stem cell transplantation is unknown and the efficacy of our transplantation technique cannot be deduced from our small population. With intracoronary administration, fewer than 5\% of infused stem cells are eventually retained in the infarcted myocardium.\textsuperscript{34} However, intracoronary administration of stem cells is a promising technique\textsuperscript{4,5,11} and clinical trials are assessing its efficacy in various settings. The safety issue, therefore, is of crucial importance for this methodology, particularly when mesenchymal cells are used for myocardial regeneration.\textsuperscript{6,9,35} The role of the preceding angioplasty and stent deployment has not been controlled in our results.

Our data provide some evidence that intracoronary administration of MSCs and endothelial progenitors do not appear to carry any arrhythmogenic potential. Of course, further studies are certainly needed on this important clinical issue.

References


