Intracoronary infusion of progenitor cells is not associated with aggravated restenosis development or atherosclerotic disease progression in patients with acute myocardial infarction

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Aims Experimental and clinical pilot studies suggest that intracoronary progenitor cell infusion can improve left ventricular function and remodelling after acute myocardial infarction (AMI). Since progenitor cells are also known to be involved in restenosis development and atherosclerosis progression, an increased restenosis rate may be a risk of intracoronary cell therapy.

Methods We performed a retrospective study to compare quantitative angiographic measurements of the infarct target vessel in 83 patients with AMI treated with bare metal stent PCI (matched control) and in 83 patients receiving additional intracoronary progenitor cell infusion at a mean of 5 days post-AMI stent PCI and after 4 months.

Results The late loss as a measure of neointima formation was similar between the control and the cell-treated group at follow-up (0.9 ± 0.8 vs. 0.9 ± 0.7 mm, P = 0.9). Moreover, restenosis rate was comparable in both groups (35% control vs. 27% cell-treated group, P = 0.2). Multivariable analysis excluded cell therapy as an independent significant predictor of increased late loss (P = 0.4), whereas acute gain (P = 0.012) and diabetes mellitus (P = 0.002) were independent predictors of late loss. Finally, in the cell-treated group, target vessel revascularization rate remained at 28.9% during a median of >3 years of follow-up, thus excluding an effect on atherosclerotic disease progression.

Conclusion In patients with AMI successfully treated with bare metal stent PCI, additional intracoronary progenitor cell infusion does not lead to an increased neointima formation within the implanted stent within 4 months or aggravation of atherosclerotic disease progression.

KEYWORDS
Acute myocardial infarction; Restenosis; Cell therapy

Introduction

Recent experimental studies suggesting that bone marrow-derived or blood-derived progenitor cells might contribute to enhanced neovascularization of ischaemic myocardium,1,2 and functional improvement of freshly infarcted hearts3–5 led to the initiation of increasing numbers of clinical trials investigating the effects of selective intracoronary infusion of progenitor cells to improve cardiac function in patients with acute myocardial infarction (AMI).6–10 However, experimental studies have also demonstrated that bone marrow-derived progenitor cells might contribute to neointima formation in transplant atherosclerosis11,12 as well as to intraplaque microvessels derived from vasa vasorum,13 which might predispose to atherosclerotic lesion growth and intraplaque haemorrhage, leading to macrophage activation and plaque destabilization.14 Thus, intracoronary infusion of progenitor cells might aggravate atherosclerotic lesion progression in patients with coronary artery disease (CAD).15 Indeed, a recently published trial suggested that intracoronary infusion of G-CSF mobilized cells was associated with a significantly increased restenosis rate in patients post-myocardial infarction.16 Moreover, preliminary data indicated a significant aggravation of atherosclerotic lesion progression at the site and distal to the selective intracoronary infusion of CD133-positive endothelial progenitor cells in patients with AMI.17 Therefore, we investigated whether intracoronary progenitor cell infusion is associated with atherosclerotic lesion progression using quantitative angiographic measurements in 83 patients with AMI treated with intracoronary progenitor cell infusion compared with a matched control group of 83 patients treated for AMI at our institution.
Methods

Study population

The control group was recruited from a registry of successful bare metal stent implantation procedures starting in 1995, which was closed in 2001 prior to initiation of the stem cell program. In this registry, a total of 321 patients with an acute ST-elevation myocardial infarction (excluding patients with cardiogenic shock) were included, and patients were advised to undergo 4 months invasive follow-up, which was performed in 270 (84%) of the patients. Of those 270 patients, we selected 83 matched patients as the control population.

Of the 350 patients with an acute ST-elevation myocardial infarction successfully treated with bare metal stent implantation in our institution between November 2001 and September 2004, 83 patients fulfilled inclusion criteria18 and consented to participate in a trial of intracoronary infusion of mononuclear bone marrow- or circulating blood-derived progenitor cells within 7 days after AMI. The 1 year follow-up of 58 of those patients has been previously reported.19 AMI patients were scheduled for 4 months invasive follow-up and long-term clinical follow-up of at least 2 years was performed. There were no patients lost to follow-up.

Matching procedure

Patients were matched with respect to age, infarct vessel, number of cardiovascular risk factors, and gender. The maximum difference in age allowed between patients was ±10 years. Mean difference in age was 3 ± 2.6 years. Gender was matched in all but eight patients (96%). Infarct vessel was matched in 76% (63) of all 83 patients (81% LCA, 64% RCA). The following cardiovascular risk factors were used for the matching procedure: diabetes, hypercholesterolaemia, hypertension, positive family history for CAD, and smoking. In addition, the cardiovascular risk factors were summarized into a score, which was ±1 for the matching procedure (match 90%). Mean difference in cardiovascular risk factor score between cell-treated patients and their controls was 0.9 ± 0.8.

The ethics review board of the Hospital of the Johann Wolfgang Goethe University of Frankfurt, Germany, approved the cell therapy protocol, and the study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each patient.

Stent implantation procedure and medication

The acute reperfusion procedure and stent implantation has been described previously.19 Either 300 mg clopidogrel as loading dose (continued as 75 mg/day) or ticlopidin (250 mg twice daily) were given for at least 4 weeks after stent implantation. Intracoronary glycerol trinitrate (0.2–0.3 mg) was given at the start of the procedure, before the final angiogram and before follow-up angiography. High-pressure balloon catheters were used for implantation of bare metal stents, with inflation pressures >10 atm. If necessary, multiple stents were used for complete coverage of the culprit lesion. No drug-eluting stents were used in any patient.

Cell preparation and cell infusion procedure

The catheterization procedure for progenitor cell transplantation has been described previously.7 Briefly, after arterial puncture, patients—pre-treated with aspirin and clopidogrel—received 7,500–10,000 units of heparin, and a bolus of abciximab (0.25 mg/kg) was given in the majority of patients prior to cell therapy. Cells were infused via an over-the-wire balloon catheter advanced into the stent previously implanted during the acute reperfusion procedure and inflated with low pressure to completely block blood flow for 3 min to allow for adhesion and potential transmigration of the infused cells through the endothelium. This manoeuvre was repeated three times to accommodate infusion of the total 10 ml progenitor cell suspension, interrupted by 3 min reflow by deflating the balloon to minimize extensive ischaemia. After completion of intracoronary cell transplantation, coronary angiography was repeated to ascertain vessel patency, absence of embolization, and unimpeded flow of contrast material. Intracoronary progenitor cell therapy was performed 5.2 ± 3.7 days after the acute reperfusion procedure for AMI. Twenty-six patients received intracoronary infusion of bone marrow-derived cells, and 57 patients received circulating mononuclear progenitor cells.

Quantitative coronary angiography

Quantitative measurements were obtained by fully computerized quantitative angiography (CMS, Medis, Leiden, The Netherlands) as previously described.19 Minimal lumen diameter (MLD), reference diameter, and percent diameter stenosis were measured in identical angiographic views before PCI, immediately after stent insertion, and at follow-up. Stent restenosis was defined as a dichotomous outcome (>50% diameter stenosis within the stent at follow-up). Acute gain was defined as the difference between MLD after stent implantation and MLD before PCI. Late loss was defined as the difference between MLD after stent insertion and MLD at follow-up. In addition, any newly formed or progressed lesion within the artery distal to the site of progenitor cell infusion was documented in the progenitor cell-treated group at the time of angiographic follow-up.

Definition of events and follow-up

The clinical endpoints included myocardial infarction, target vessel revascularization (TVR), or any revascularization procedure during follow-up. Of primary interest was the clinical endpoint TVR, which was defined as repeat intervention of restenotic lesions including the target site of the stent implantation or proximal or distal in the same major coronary artery. Any revascularization was defined as the necessity for PCI or any bypass surgery during follow-up. Both TVR and any revascularization procedure were driven by the presence of symptoms of angina or a positive stress test.

Data and statistical analysis

The statistical power of the study is 0.80 to detect a clinically meaningful difference in late loss of 0.33 mm, with an α error of 0.05. Cumulative distribution curves of MLD were obtained for both groups of patients prior to and immediately after PCI, as well as at 4 months follow-up. Kolmogorov–Smirnov testing was applied to assess normality of distribution for continuous variables.

Continuous variables are presented as mean ± SD, and were compared between the different treatment groups with the non-parametric Mann–Whitney U test. Categorical variables were compared with the χ² test or Fisher’s exact test. In addition, paired non-parametric Wilcoxon testing was applied for direct comparison between the matched patients.

In order to determine independent predictors of late loss, multivariable analysis was performed using the multiple stepwise linear regression model. A forward entry stepping algorithm was used with the entry criteria probability of F (0.05). We chose an epiphenological approach and selected the following variables, which are known to be associated with increased late loss into the multivariable analysis: diabetes mellitus, acute gain, and reference lumen diameter, in addition to intracoronary cell therapy.

Statistical significance was assumed if P < 0.05 (two-sided). All statistical analysis was performed using SPSS (Version 14.0, SPSS Inc.).

Results

Baseline characteristics

The baseline clinical and procedural characteristics of the two study populations are summarized in Table 1. The two
groups were well matched with respect to age, target vessel, and cardiovascular risk factors except smoking. In contrast, baseline medical treatment (Table 2) revealed significant differences between the two treatment groups, reflecting the change of standard medication over time. Anti-platelet therapy included the use of ticlopidine or clopidogrel in addition to aspirin in the majority of patients. In addition, secondary prevention therapy using ACE-inhibitors, β-blockers, and statins was statistically more frequently used in the cell therapy group.

### Angiographic results

Table 3 summarizes the quantitative angiographic results at baseline and during follow-up. Patients treated with intracoronary progenitor cell therapy suffered more frequently from a total target vessel occlusion at the time of AMI revascularization (Figure 1A), resulting in a significantly higher mean diameter stenosis grade when compared with the standard treatment group. Immediately after stent implantation, the MLD was identical in both groups, whereas the residual stenosis grade was slightly, but not significantly, smaller in the cell therapy group. As a consequence of more frequent total target vessel occlusions, the acute gain was significantly greater in the cell-treated patients compared with the control group.

Follow-up angiography was performed after a mean of 122 ± 27 (median 120, inter-quartile range 112–130) days in both groups. There was no difference in any of the quantitative angiographic parameters between both groups (Table 3). Figure 1A illustrates the cumulative frequency curves of MLD pre-, immediately post-, and 4 months after stent implantation for the two treatment groups, documenting that late lumen loss, as shown in Figure 1B, did not differ between the two groups.

When using the categorical criterion of ≥50% diameter stenosis at follow-up, restenosis rate was not significantly different between both groups. In none of the cell-treated patients was there development of a new lesion distal to the site of progenitor cell infusion. The quantitative angiographic results did not differ when the cell-treated patients were analysed separately according to the type of cells received.

In order to identify independent predictors of late loss, a multivariable analysis including established predictors of increased late loss after coronary stent implantation was performed. As shown in Table 4, the additional intracoronary progenitor cell infusion procedure is not independently associated with an increased late loss during follow-up (P = 0.43). Only the presence of the classical determinants diabetes mellitus and an increased acute gain after stent implantation independently predicted the extent of neointima formation.

### Clinical events during follow-up

Table 5 summarizes the occurrence of a clinical endpoint in the two patient groups, except death (since patients were included only if they had a complete angiographic follow-up). Importantly, there were no significant differences in myocardial infarction and subacute stent
thrombosis, as well as in both TVR and any revascularization procedure in patients undergoing intracoronary progenitor cell infusion compared to patients of the control group, documenting the safety in clinical outcome with respect to TVR in the cell-treated group. Thus, intracoronary progenitor cell infusion clearly does not aggravate restenosis development or disease progression within the infused coronary artery.

Finally, extended clinical follow-up of a median of 3 years of the cell-treated patients revealed that only 28.9% of these patients required an additional TVR procedure (Figure 2), thus excluding a potential long-term effect of intracoronary cell administration on atherosclerosis disease progression.

**Discussion**

The data presented in our study describe the results of quantitative angiographic analysis of the infarct-related lesion in the largest cohort of patients undergoing intracoronary infusion of progenitor cells for AMI so far reported. By retrospective comparison with a matched, historical patient population, our results demonstrate that intracoronary infusion of progenitor cells does not aggravate restenosis development or disease progression within the infused coronary artery.

Table 3: Angiographic characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control n = 83</th>
<th>Cell therapy n = 83</th>
<th>P-value between groups</th>
<th>P-value between pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prior to PCI</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>MLD (mm)</td>
<td>0.51 ± 0.5</td>
<td>0.32 ± 0.5</td>
<td>0.004</td>
<td>0.010</td>
</tr>
<tr>
<td>Stenosis (%)</td>
<td>83.0 ± 16</td>
<td>90.0 ± 15</td>
<td>0.002</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>Immediately after PCI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference vessel diameter (mm)</td>
<td>3.23 ± 0.7</td>
<td>3.16 ± 0.5</td>
<td>0.77</td>
<td>0.21</td>
</tr>
<tr>
<td>MLD (mm)</td>
<td>2.74 ± 0.5</td>
<td>2.78 ± 0.4</td>
<td>0.45</td>
<td>0.78</td>
</tr>
<tr>
<td>Stenosis (%)</td>
<td>14.7 ± 11</td>
<td>11.0 ± 11</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>Acute gain (mm)</td>
<td>2.23 ± 0.7</td>
<td>2.46 ± 0.6</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>At 4 months follow-up</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference vessel diameter (mm)</td>
<td>3.06 ± 0.7</td>
<td>3.05 ± 0.6</td>
<td>0.99</td>
<td>0.68</td>
</tr>
<tr>
<td>MLD (mm)</td>
<td>1.84 ± 0.9</td>
<td>1.88 ± 0.8</td>
<td>0.81</td>
<td>0.67</td>
</tr>
<tr>
<td>Stenosis (%)</td>
<td>39.2 ± 25</td>
<td>38.0 ± 23</td>
<td>0.98</td>
<td>0.68</td>
</tr>
<tr>
<td>Late loss (mm)</td>
<td>0.89 ± 0.8</td>
<td>0.90 ± 0.7</td>
<td>0.87</td>
<td>0.91</td>
</tr>
<tr>
<td>Late loss index</td>
<td>0.46 ± 0.5</td>
<td>0.36 ± 0.3</td>
<td>0.20</td>
<td>0.09</td>
</tr>
<tr>
<td>Restenosis rate (%)</td>
<td>35</td>
<td>27</td>
<td>0.16</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Table 4: Multivariable analysis for predictors of late loss

<table>
<thead>
<tr>
<th></th>
<th>Non-standardized coefficient B</th>
<th>P-value</th>
<th>95% CI for B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus</td>
<td>0.43</td>
<td>0.002</td>
<td>0.16–0.70</td>
</tr>
<tr>
<td>Acute gain</td>
<td>0.26</td>
<td>0.012</td>
<td>0.06–0.46</td>
</tr>
<tr>
<td>Reference diameter post-PCI</td>
<td>$-0.05$</td>
<td>0.67</td>
<td>$-0.26–0.17$</td>
</tr>
<tr>
<td>Cell therapy</td>
<td>$-0.09$</td>
<td>0.43</td>
<td>$-0.33–0.14$</td>
</tr>
</tbody>
</table>

Adjusted $R^2 = 0.10$, significance (ANOVA) $P = 0.003$. 

Figure 1 (A) Cumulative frequency of MLD immediately prior to and after stent PCI as well as at follow-up for the control group (blue line) and the cell therapy group (red line). (B) Cumulative frequency of late lumen loss for the control group (blue line) and the cell therapy group (red line).
Intracoronary infusion of progenitor cells

Table 5 Clinical endpoints at 4 months

<table>
<thead>
<tr>
<th></th>
<th>Control n = 83</th>
<th>Cell therapy n = 83</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial infarction (n, %)</td>
<td>4 (5)</td>
<td>3 (4)</td>
<td>1.00</td>
</tr>
<tr>
<td>Subacute stent thrombosis TV (n, %)</td>
<td>1 (1)</td>
<td>2 (2)</td>
<td>1.00</td>
</tr>
<tr>
<td>TV revascularization (n, %)</td>
<td>30 (36)</td>
<td>19 (23)</td>
<td>0.09</td>
</tr>
<tr>
<td>Any revascularization (n, %)</td>
<td>36 (43)</td>
<td>30 (36)</td>
<td>0.43</td>
</tr>
</tbody>
</table>

TV, target vessel.

Figure 2 Event-free survival of infarct (=target) vessel revascularization of the cell-treated patients.

with increased cardiovascular events including the necessity for repeated coronary revascularization procedures. Thus, intracoronary progenitor cell application does not aggravate atherosclerotic disease progression in patients after AMI.

A recently published study suggesting the occurrence of increased restenosis development following intracoronary infusion of G-CSF mobilized progenitor cells has ignited a fierceful discussion with respect to the safety of progenitor cell treatment in patients after AMI. Moreover, Bartunek and co-workers even suggested that infusion of bone marrow-derived mononuclear cells may not only increase restenosis development, but may also accelerate atherosclerotic lesion progression distal to the site of progenitor cell infusion.

How can these results be reconciled with the data described in the present report? First of all, Kang et al. treated patients with G-CSF for 5 days prior to percutaneous intervention of a high-grade coronary lesion. Although G-CSF is known to have anti-inflammatory effects, especially in bacteremia and sepsis, in the specific setting of an acute coronary syndrome and myocardial infarction, the potent pro-inflammatory effects of G-CSF may be of stronger relevance. Thus, it is not surprising that stent implantation with or without concomitant progenitor cell infusion was associated with increased restenosis rate. In fact, it is well established that inflammatory activation is the most important determinant of increased restenosis development following PCI. Secondly, Bartunek and co-workers not only obtained large amounts of bone marrow aspirate (>180 mL) under general anaesthesia, which resulted in profound CRP increases, but—more importantly—used an irreversibly attached murine antibody to purify CD133-positive cells from the mononuclear cell fraction derived from bone marrow aspirate. The murine antibody was not removed prior to infusion of the cells into the human coronary circulation. Thus, although the authors claim that no human anti-mouse antibodies were detected in the systemic circulation of the patients following infusion of progenitor cells, it is most likely that the mouse antibody attached to the infused human CD133 mononuclear cells might have caused an inflammatory reaction at and distal to the site of intracoronary infusion, which contributed to the observed acceleration of atherosclerotic disease progression. Taken together, it may be well rationalized that the study design itself as well as the specific properties of the re-infused cells is responsible for the observed aggravation of the atherosclerotic process rather than the intracoronary infusion of progenitor cells.

Indeed, two very recent studies did not demonstrate any increase in coronary arterial luminal narrowing following G-CSF administration in patients undergoing PCI for AMI prior to G-CSF treatment. Moreover, experimental studies demonstrated that G-CSF administration for mobilization of endothelial progenitor cells accelerated re-endothelialization of denuded vessels. Likewise, none of the small scale studies using intracoronary infusion of unselected mononuclear cells derived from the bone marrow reported exaggerated restenosis rates during follow-up in patients with AMI. In fact, there is ample experimental evidence that infusion of bone-marrow or blood-derived progenitor cells accelerates re-endothelialization of injured arteries, reduces neointima formation after vascular injury, and rejuvenates the vascular wall in aged animals. Indeed, we have recently shown that elevated levels of circulating endothelial progenitor cells independently predict a beneficial outcome in patients with CAD, providing proof-of-concept for the existence of endogenous vascular repair capacity of circulating endothelial progenitor cells.

The major limitation of the present study is its retrospective design, as well as the control patient pool with an angiographic follow-up rate of 84%. Although patients were matched with respect to age, gender, infarct vessel, and number of cardiovascular risk factors, there remained differences in clinical and angiographic variables at baseline between the two patient populations. The progenitor cell-treated patients were more frequently smokers and had less extensive CAD. On the other hand, they had significantly larger myocardial infarctions with reduced left ventricular ejection fractions. Although reduced left ventricular ejection fraction is well established to be associated with a worse outcome following coronary stent implantation, the impact of smoking on restenosis development is not yet clear. However, on multivariate analysis, only the risk factor diabetes as well as the acute gain remained independent predictors of an increased late loss in the total patient population. Thus, it is unlikely that the different clinical
baseline characteristics may have confounded our results. Likewise, during the last decade, antiplatelet therapy has changed from ticlopidine to clopidogrel in combination with aspirin, and secondary prevention with ACE-inhibitors, β-blockers, and statins was maximized in the cell-treated patient group. However, none of these differences due to changes in pharmacological treatment over time appeared to have impacted on the results comparing the cell-treated group with a historical control group. The larger acute gain associated with smaller residual stenosis after stent implantation as well as the maximized secondary prevention medications might have contributed to the slightly reduced TVR rates observed in the cell-treated group compared with the historical control group. Finally, the major strength of the present study is the unprecedented long duration of follow-up with a median observation period of > 3 years in the cell-treated patients cohort. The lack of an increase in revascularization rates during 3 years of follow-up clearly excludes a potential long-term effect on atherosclerotic disease progression.

In summary, the results of the present study do not support any significant role for intracoronary progenitor cell infusion to aggravate atherosclerotic lesion progression in patients post-myocardial infarction. In addition, the low-pressure balloon inflation within a previously implanted stent—necessary for intracoronary cell transplantation—in patients 3–7 days following reperfusion therapy for AMI does not aggravate restenosis development even when bare metal stents are used. Thus, the potential beneficial effects of intracoronary progenitor cell infusion on cardiac function in patients with AMI do not appear to be compromised by an acceleration of the atherosclerotic disease process.

Conflict of interest: V.S. reports to have received consulting fees from Guidant and AstraZeneca and lecture fees from Pfizer, Novartis, Merck Sharp & Dohme, Lilly, Boehringer-Ingelheim, Sanofi-Aventis, and Boston Scientific. S.D. reports to have received consulting fees from Guidant and Grynzee and lecture fees from Medtronic. A.M.Z. reports to have received consulting fees from Guidant. S.D. and A.M.Z. report that they are cofounders of L2cure, a for-profit company focused on regenerative therapies. They serve as scientific advisers and are shareholders.

References


