SCIENTIFIC LETTER

Feasibility of trans-endocardial cell transplantation in chronic ischaemia

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Cell transplantation is currently being explored as a novel therapeutic option for patients with chronic myocardial ischaemia. The main goal of bone marrow cell transplantation is to augment blood flow in ischaemic myocardium through induction of angiogenesis. Improved collateral perfusion and enhanced left ventricular function have already been documented in animal models with chronic myocardial ischemia.¹ Until now, most clinical studies were carried out in patients with acute myocardial infarction. Only two studies containing ≤ 10 patients assessed the safety and feasibility of autologous bone marrow cell transplantation in patients with angina and ischaemia.^{2,3} Further studies are needed to confirm the safety, feasibility and effectiveness reported in these pilot trials.

The present phase I/II study tested the feasibility, safety and clinical effects of trans-endocardial transplantation of autologous bone marrow-derived mononuclear cells in patients with drug-refractory angina and myocardial ischaemia. Moreover, we aimed to determine whether cell transplantation is associated with improved myocardial function and perfusion by means of technetium-99m tetrofosmin gated single photon emission computed tomography (SPECT).

METHODS

Patients with ischaemia on technetium-99m tetrofosmin SPECT and with Canadian Cardiovascular Society (CCS) class III or IV angina pectoris (despite maximal tolerated medical treatment) without options for conventional revascularisation were included in the study. An independent expert panel that reviewed the angiograms determined the ineligibility for surgical or percutaneous revascularisation. Seventeen patients fulfilled the inclusion criteria and were considered for inclusion. Exclusion criteria were acute myocardial infarction < 6 months before enrolment, history of malignancy, renal dysfunction or unexplained haematological or biochemical abnormalities. The institutional ethics committee approved the protocol, and all patients gave informed consent.

At baseline, CCS class was graded by an independent investigator. A bicycle exercise test and 24 h Holter monitoring were performed to evaluate exercise capacity and the occurrence of arrhythmias. Technetium-99m tetrofosmin gated SPECT was carried out to assess left ventricular function and myocardial perfusion.

Bone marrow was harvested from the iliac crest under local anaesthesia, on the morning of the transplantation procedure. Mononuclear cells were isolated by Ficoll density gradient. Trans-endocardial cell injections were targeted at myocardial regions with stress-inducible ischaemia (on SPECT) and injected with the NOGA system (Biosense-Webster, Waterloo, Belgium). After the procedure, heart rhythm monitoring was started and laboratory markers of myocardial necrosis were collected. Patients continued their routine drug intake after

cell transplantation. Before discharge, two-dimensional echocardiography was carried out to exclude post-procedural pericardial effusion.

At 3 months and 6 months follow-up, CCS class, exercise capacity (bicycle test) and the occurrence of arrhythmias (24 h Holter) were assessed. Gated SPECT reassessed left ventricular function and myocardial perfusion at 3 months.

For SPECT imaging, a two-day stress-rest protocol was used. The stress protocol included a bicycle exercise test or 0.14 mg/kg/min adenosine infusion (6 min) in patients unable to exercise ($n = 7$). At 1 min before termination of the test, 500 MBq technetium-99m tetrofosmin was injected intravenously. On the second day, electrocardiograph-gated resting images were obtained using 500 MBq technetium-99m tetrofosmin. Imaging was carried out with a triple-head SPECT camera system (GCA 9300/HG, Toshiba Corp, Tokyo, Japan). Reconstruction yielded standard short-axis and long-axis projections perpendicular to the heart axis, which were adjusted for peak myocardial activity (100%). SPECT at 3 months was performed under identical conditions as at baseline. Two experienced observers blinded to all clinical data reviewed the SPECT images. Perfusion was assessed using segmental tracer activity (17-segment model) and expressed as percentage of maximum. Perfusion defects on stress images were considered present when tracer activity was $< 75\%$. When significant fill-in ($> 10\%$) of perfusion defects was observed on the resting images, segments were classified as ischaemic. Left ventricular ejection fraction (LVEF) and regional wall motion was calculated using QGS software (Cedars-Sinai Medical Center, Los Angeles, California).⁴

Results are presented as mean (standard deviation (SD)). Data were compared using paired Student's *t* test or χ^2 analysis with Yates correction where appropriate. A *p* value < 0.05 was considered significant.

RESULTS

Fifteen patients (12 men, mean (SD) age 67 (7) years) received bone marrow cell transplantation. Patients' medications included nitrates (15/15), β blockers (15/15) and calcium channel blockers (12/15). Ten patients had a previous myocardial infarction (4 anterior, 1 lateral, 5 inferior). Conventional revascularisation was ineligible owing to diffuse coronary artery disease with poor target vessels. Medication remained unchanged during the study period.

Patients were successfully injected with $92 (42) \times 10^6$ mononuclear cells (10.5 (2) injections). Sustained ventricular tachycardia was not observed during left ventricle mapping, cell transplantation or hospitalisation. Laboratory values did

Abbreviations: CCS, Canadian Cardiovascular Society; LVEF, left ventricular ejection fraction; SPECT, single photon emission computed tomography

Figure 1 Scatter plot showing individual left ventricular ejection fractions (LVEF) before (Pre) and 3 months after cell transplantation (Post). The solid line represents the line of identity.

not show infarction. Pre-procedural creatinine kinase was similar to in-hospital peak creatinine kinase (103 (63) v 128 (66) U/l; $p = \text{NS}$) and in-hospital peak troponin T ranged from 0.07 to 0.61 $\mu\text{g/l}$ (cut-off value for infarction $>0.8 \mu\text{g/l}$). Two-dimensional echocardiography excluded post-procedural pericardial effusion. Repetitive 24-h Holter recordings did not show sustained ventricular tachycardia. During the 6 months follow-up period, no major adverse events occurred.

At 3 months, CCS class improved in 13 of 15 patients: 4 patients showed an improvement of 2 CCS classes and 9 patients improved by 1 CCS class. Three patients exhibited additional improvement in CCS class at 6 months, whereas 4 patients showed initial improvement at 3 months followed by worsening at 6 months.

Although exercise capacity remained unchanged (100 (30) v 105 (27) W at 3 months and 106 (28) W at 6 months; both $p = \text{NS}$ v baseline), the number of patients who stopped the test owing to angina decreased from 12 to 6 at 3 months ($p = 0.03$) and to 5 at 6 months ($p < 0.01$) out of a total of 15 patients. In addition time to onset of significant ST-segment changes increased from 4.8 (3.1) to 5.6 (3.3) min at 3 months ($p = 0.04$) and to 5.8 (2.7) min at 6 months ($p < 0.01$).

LVEF increased from 50 (13)% to 58 (15)% at 3 months follow-up ($p < 0.01$). In particular, LVEF improved by $\geq 5\%$ in 11 of 15 patients (fig 1). Mean segmental wall motion improved from 6.35 (1.87) to 7.19 (2.1) mm at 3 months ($p = 0.02$).

Of the 255 segments assessed, 83 (33%) showed ischaemia at baseline as compared with 30 (12%) at 3 months follow-up ($p < 0.01$). Cell transplantation, in particular, improved stress perfusion, whereas resting perfusion remained unchanged. The proportion of segments with $\geq 75\%$ tracer uptake (normal perfusion) during stress increased from 156 of 255 (61%) at baseline to 206 of 255 (81%) at 3 months ($p < 0.01$), whereas the proportion of segments with $\geq 75\%$ tracer uptake

at rest remained unchanged (227/255 (89%) v 234/255 (92%); $p = \text{NS}$).

DISCUSSION

The present phase I/II study in patients with severe angina and myocardial ischaemia, who were trans-endocardially transplanted with autologous bone marrow-derived mononuclear cells, showed excellent safety and feasibility results. The procedure was well tolerated and no procedure-related side effects occurred. No major adverse event occurred during the 6 months follow-up period. Furthermore, major clinical improvements were observed: anginal symptoms reduced, left ventricular function increased and myocardial perfusion augmented.

As the current study comprises no control group, the observed changes cannot be unambiguously attributed to bone marrow cell transplantation. Other contributing factors might be placebo effect, spontaneous improvement and medical treatment. However, it is rather rare that left ventricular function and myocardial perfusion improve without intervention, and cardiovascular drugs remained unchanged in all patients. The 6 months follow-up period can be considered a limiting factor: the long-term effects of cell transplantation remain to be determined. Additional randomised, placebo-controlled studies are warranted to further pursue the strategy of cell transplantation in patients with angina and chronic ischaemia.

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