European Heart Journal (2005) **26**, 1188–1195
doi:10.1093/eurheartj/ehi159

Clinical research

Percutaneous trans-coronary-venous transplantation of autologous skeletal myoblasts in the treatment of post-infarction myocardial contractility impairment: the POZNAN trial†

Tomasz Siminiak[1]*, Dorota Fiszer[2], Olga Jerzykowska[1], Beata Grygielska[2], Natalia Rozwadowska[2], Piotr Kałmucki[1], and Maciej Kurpisz[2]

[1] *University School of Medical Sciences, Department of Cardiology, District Hospital, ul. Juraszow 7/19, PL 60-479, Poznań, Poland*
[2] *Institute of Human Genetics, Polish Academy of Sciences, Poznań, Poland*

Received 2 August 2004; revised 30 December 2004; accepted 6 January 2005; online publish-ahead-of-print 10 March 2005

KEYWORDS
Myocytes;
Heart failure;
Cells;
Catheters;
Myocardial infarction

Aims Several experimental studies and the initial clinical experience have shown that autologous skeletal myoblast transplantation into the area of post-infarction left ventricular injury results in an increase in segmental contractile performance. This phase I clinical trial was designed to assess the feasibility and safety of autologous skeletal myoblast transplantation performed via a percutaneous trans-coronary-venous approach in patients with post-infarction left ventricular dysfunction.
Methods and results Ten patients with heart failure and presence of an akinetic or a dyskinetic post-infarction injury with no viable myocardium were included in the study. Skeletal myoblasts were obtained from a biopsy specimen and grown in cell culture. Patients were treated with prophylactic amiodarone infusion before and during the procedure, except one patient. Skeletal myoblast transplantations were performed uneventfully in nine patients using the TransAccess® catheter system under fluoroscopic and intravascular ultrasound guidance. In one patient, the procedure was not performed because of the inability of appropriate coronary sinus guiding wire positioning across venous valve. In five patients, the anterior interventricular vein and in four patients, the middle cardiac vein were used to access the myocardium. Two to four intramyocardial injections 1.5–4.5 cm deep were performed in each patient, delivering up to 100 million cells in 0.4–2.5 mL of saline. During 6 months follow-up, New York Heart Association class improved in all patients and ejection fraction increased 3–8% in six out of nine cases.
Conclusion These data suggest the feasibility and procedural safety of myoblast transplantation performed via the trans-coronary-venous approach using the TransAccess catheter system.

* Corresponding author. Tel: +48 602 217 202; fax: +48 61 8212319.
E-mail address: tomasz.siminiak@usoms.poznan.pl

† Presented in part as a Late Breaking Clinical Trial at the 53rd Annual Scientific Session, American College of Cardiology, March 2004, New Orleans, LA, USA.

Introduction

Post-myocardial infarction congestive heart failure remains to be a major clinical problem, despite recent advances in the medical and surgical treatment of acute coronary syndromes. Development of heart failure in survivors from acute myocardial infarction (MI) is known to involve myocardial cell loss in the area supplied by the infarct-related artery and subsequent formation of fibrous tissue that is unable to contract. Routinely performed revascularization procedures enable recovery of contractile function only in the areas of hibernating myocardium, having a certain amount of viable, reversibly injured myocytes, which are capable of restoration of performance as a result of increased coronary perfusion. Formation of fibrous tissue as a replacement of myocardial cells lost during the acute phase of MI leads to decreased systolic function, left ventricular remodelling, aneurysm formation, and progression of congestive heart failure. Cardiac transplantation may be an option in selected patients, but because organ shortage, its practical use is limited.

Both pre-clinical data and initial clinical experience obtained in the recent years indicate that restoration or improvement of the left ventricular contractile performance can be observed as a result of supplementing the area of infarcted myocardium with precursor cells that can differentiate into myocytes.[1–6] Several cell types have been investigated as possible substrates for myocardial replacement therapy. Potential use of embryonic stem cells or foetal cardiomyocytes raises serious issues of political, ethical, and even religious nature and, therefore, is far from consideration for clinical applications. Experimental data suggest that autologous bone marrow stem cells when injected into healthy or recently infarcted myocardium differentiate into myocytes and/or fuse with existing cardiomyocytes, but their implantation within post-infarction scar may result in their differentiation into fibroblasts.[3,4] Therefore, direct myocyte precursors, myoblasts, are under investigation for the enhancement of contractile function within the area of fibrous post-infarction scar. Data[7] obtained on an experimental model of post-infarction injury have shown that foetal cardiomyocytes and skeletal myoblasts (satellite cells) are similarly effective in improving left ventricular haemodynamic parameters including contractile function. Implantation of both cell types resulted in a significant increase in ejection fraction, whereas injection of cell culture medium alone resulted in a decrease in left ventricular ejection fraction (LVEF). In addition, histological signs of differentiation of both cell types into mature myotubes have been noted.[7]

Data from experimental studies have encouraged Menasché *et al.*[8] to perform the first autologous skeletal myoblast transplantation in a patient, a survivor from MI, during coronary artery bypass grafting (CABG). This was shortly followed by an independent phase I clinical study initiated at our centre.[9,10] Phase II clinical studies evaluating the efficacy of autologous myoblast transplantation performed at the time of CABG are ongoing, but despite careful patient selection, it may be problematic to assess the effect on contractility of transplanted cells independently of the positive effects of revascularization. In addition, single case reports of percutaneous myoblast transplantations were published by our group[11] and others.[12]

We report the procedural feasibility and safety results of the first phase I clinical trial on percutaneous transvenous autologous myoblast transplantation into an area of post-infarction injury. The procedures were performed as a sole therapy, that is, without concomitant revascularization, using a catheter system (TransAccess®, TransVascular Inc., Menlo Park, CA, USA), allowing intramyocardial injections performed via the cardiac venous approach under intravascular ultrasound (IVUS) guidance.

Methods

The primary endpoints of this phase I clinical trial were feasibility and safety. Feasibility was defined as the possibility of expansion of the target number of cells within 4 weeks of *in vitro* culture and the ability to establish a cell culture and transportation system to the catheterization laboratory, allowing delivery of autologous myoblasts suspensions having >90% viability and purity as well as the possibility to perform percutaneous intramyocardial cell injections into the akinetic/dyskinetic segments of the left ventricle. Safety referred to any adverse events related to skeletal muscle biopsy and/or transplantation of cells into the myocardium, with special attention to detect arrhythmia as well as the use of the catheter system. The secondary endpoint was the efficacy assessed by New York Heart Association (NYHA) heart failure class evaluation and two-dimensional echocardiographic analysis of the left ventricular function before the procedure and during 6 months follow-up period.

Patients studied

The inclusion criteria for the study were as follows: documented MI occurring a minimum of 3 months before the procedure; age <65 years; ejection fraction >25% and <40%; one to three akinetic or dyskinetic segments seen on echocardiography and lack of myocardial viability on dobutamine echocardiography; heart failure NYHA class 2 or 3; and good coronary artery blood flow into the area of post-infarction injury as a result of previous revascularization or development of collateral flow. The main exclusion criteria consisted of episodes of unstable ischaemia after the infarction onset or other causes requiring urgent revascularization, known inflammatory disease, renal insufficiency, history of stroke, peripheral muscular dystrophy, and inability to fully understand the protocol and the experimental nature of the procedure.

Transthoracic two-dimensional echocardiography was performed to screen patients, survivors from acute MI presenting with heart failure NYHA class 2 or 3. Global left ventricular ejection fraction (LVEF) (%) was calculated from LV end-diastolic volume (mL) and LV end-systolic volume (mL) using the Simpson's rule. Regional contractile function was assessed semiquantitatively after division of the left ventricle into 16 segments, according to the recommendations of the American Society of Echocardiography.[10] After optimization of endocardial visualization on three parasternal short-axis views at three levels of the left ventricle, as well as apical two- and four-chamber views, segmental thickening was blindly graded by two experienced echocardiographers. Patients having a documented history of MI and with an akinetic or a dyskinetic area within the left

ventricle, were screened by means of dobutamine stress echocardiography and included in the study when no viable myocardium was detected. Absence of viability in the akinetic/dyskinetic area was assumed if no change in segmental contractility after administration of 5, 10, and 15 μg/kg per min of dobutamine with no increase in the heart rate >10 beats/min has been observed. Patients were enrolled into the study group when both blinded examiners clearly agreed upon the lack of signs of viable myocardium.

As typical phase I study requires inclusion of 10 patients, 10 consecutive patients fulfilling the inclusion criteria, who received extensive information on the procedure and subsequently gave their written informed consent, were enrolled. Eight patients from the study group underwent previous revascularization, and in two cases the infarct area was supplied by well-developed collateral circulation. The minimum thickness of the left ventricular wall at the site of post-infarction myocardial akinesia/dyskinesia was 3 mm. Two patients from the study group had previously had an implantable cardioverter defibrillator (ICD) implanted. All patients received beta-blockers, ACE-inhibitors, and aspirin.

The investigation was approved by the Bioethical Committee of the University School of Medical Sciences in Poznan.

Cell preparation

A biopsy specimen of skeletal muscle was obtained from each patient, and myoblasts were isolated and grown in cell *in vitro* culture, as previously described.[8,9,13] Briefly, a biopsy sample ~1 cm^3 was obtained from the vastus lateralis by an incision performed under local anaesthesia. The biopsy specimen was cleared of remains of fatty and connective tissue and minced into ~1 mm^3 pieces in Hanks' solution. After centrifugation (1200 r.p.m., 10 min at 20°C), the supernatant was suspended in culture medium. The tissue was digested with collagenase I (0.01%, 15 min at 33°C) and then filtered in sterile conditions through a nylon filter of 80 μm. Digestion and filteration were repeated, and cells resuspended in culture medium (Dulbecco MEM) supplemented with 20% FCS. The cells were taken in a logarithmic phase of expansion, washed, and resuspended in physiological saline in order to avoid provoking foreign antigens contained in culture medium. Cell viability as assessed by 1% Trypan Blue exclusion always exceeded >95%. Myoblasts were assessed by histochemistry using anti-CD 56 antibody and anti-desmin antibody, which indicated altogether >65% of myoblasts contained in the cell suspension. After *in vitro* expansion, serum was withdrawn and albumin added for proof of myotube formation. Myosin heavy chain staining provided evidence for almost 100% of cells positive for MHC.[13] As a result of 3 weeks cell culture, up to 10^8 myoblasts were obtained. Two microbiological controls of the cell culture were performed and were both negative. No effect on viability as assessed by Trypan Blue staining was observed after passaging of the cells through the 27-gauge microinfusion catheter.

Technique of cell delivery

The procedures of percutaneous trans-coronary-venous cell transplantation were performed as previously described.[11,15] After introducing 6-French (F) arterial and 11F venous femoral sheaths, coronary angiography was performed, allowing prolonged recording (Axiom Artist, Siemens, Germany) of the venous flow to determine coronary venous anatomy, anomalies, and coronary sinus location. The coronary sinus was engaged with a 7F SIM1 catheter (Cook, USA), and over it a 10F CS guiding catheter (TransVascular) was introduced into the coronary sinus. A balloon located at the tip of the guiding catheter was briefly inflated under low pressure to slow down the coronary sinus blood outflow, and a retrograde dye injection was performed via the guiding catheter in order to visualize the coronary venous system. The SIM1 catheter was replaced by an exchange length, 0.014 in. Hi Torque Floppy guide wire (Guidant, Temecula, CA, USA), and the wire was advanced into the anterior interventricular coronary vein via the great cardiac vein or into the middle cardiac vein. If the retrograde dye injection through the guiding catheter was not able to opacify the distal part of the target coronary vein because of the presence of venous valves, a sub-selective catheterization of the target vein over the guide wire was performed. After appropriate positioning of the guiding catheter and the guide wire, the TransAccess catheter (TransVascular) was advanced over the guide wire into the target coronary vein. The TransAccess catheter (*Figure 1*) is a 6.2F, monorail, composite catheter system combining a phased-array IVUS (compatible with Volcano IVUS system, Volcano Therapeutics, Rancho Cordova, CA, USA) and a pre-shaped 24-gauge nitinol needle. As the TransAccess system was placed in the target coronary vein, the intravascular orientation was performed using the corresponding artery, pericardium, and ventricular myocardium as landmarks with IVUS imaging. After confirmation of the appropriate TransAccess catheter position within the coronary vein by IVUS imaging and after verification of the depth of the catheter system in the vein by fluoroscopy, the nitinol needle was extended 3–7 mm into the myocardium. A 27-gauge microinfusion catheter (IntraLume™, TransVascular Inc.) was then advanced through the needle, centimetres into the myocardium with simultaneous injection of cell suspension into the target area.

Cell suspensions containing up to 10^8 myoblasts in 0.4–2.5 mL of saline were injected in two to four channels produced by the IntraLume microcatheter. Cells were washed directly before cell implantation in saline and resuspended in the saline volume suggested by the operator. The injections were performed towards the centre of the scar area during the advancement

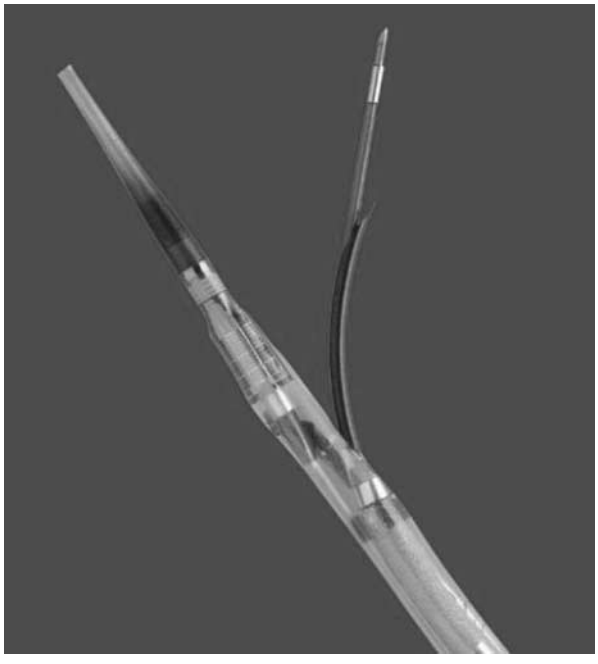

Figure 1 TransAccess (TransVascular Inc.) catheter tip. Note the IVUS transducer at the end of the catheter and pre-shaped needle with IntraLume microcatheter tip advanced through the needle.

of the IntraLume microcatheter into the myocardium to minimize the tissue injury. The injection channels were 1.5–4.5 cm deep, as assessed by the fluoroscopic distance measurements. The duration of the procedures was 55–178 min (mean 97 min), the fluoroscopy time 14–40 min (mean 25 min), and the volume of the contrast medium given did not exceed 250 mL. The protocol did not allow injections of contrast medium to ensure that the tip of microinfusion catheter was within the left ventricular wall, as the contrast medium may affect cell viability/engraftment. However, observing the resistance and force applied to the syringe during injections, it could be assumed that the whole volume of cell suspension has been delivered into the myocardium.

Safety issues

Muscle biopsy and myoblast culture were successfully completed in 12 patients. Two patients were not admitted to the study for the following reasons: one patient experienced ventricular tachycardia (VT) accompanied by chest pain just prior to attempting the procedure, despite prior amiodarone infusion; and the cells of the very first patient expired (time post-harvest from culture) prior to the procedure being attempted—cells were resuspended in saline prior to injection.

Successful intramyocardial injections of the cell suspensions into the target area of the left ventricle were performed in 9 out of 10 patients; procedural details are given in *Table 1*. In one patient, we were unable to advance the guide wire past a valve in the great cardiac vein and, therefore, the procedure was abandoned. In five patients, the anterior interventricular vein (*Figure 2*) and in four patients, the middle cardiac vein (*Figure 3*) was used to access to myocardium with the TransAccess catheter system. In each of the nine patients, two to four intramyocardial injections were performed delivering cell suspensions in 0.6–2.5 mL of saline. Each of intramyocardial injections resulted in formation of channels 1.5–4.5 cm deep within the myocardial injured area, as the IntraLume™ catheter was advanced into the myocardium during slow injection of cell suspension. No injection-related chest pain was observed. The target region has been reached in all nine successful cases.

No signs of myocardial ischaemia were detected during the procedure, except of patient number 5, reporting a brief episode (∼15 s) of chest pain at the time of the catheter advancement deeply into the anterior interventricular vein, possibly due to decreased venous blood outflow, caused by the presence of the catheter system in the distal, narrow segment of the vein. No significant rise in troponin T or CK were observed in all nine patients undergoing the procedure. No venous extravasation was observed after removal of the injection catheter.

Nine patients from the study group were treated with prophylactic amiodarone infusions started the day before the procedure (1200 mg/24 h i.v. the first day; 600 mg/24 h i.v. during the procedure) and kept on oral amiodarone (initiated day 1 after the procedure) during the next 2–3 weeks. One patient from the study group, reporting allergic reaction to amiodarone in the past and having ICD implanted, was not treated with prophylactic amiodarone.

Only single ventricular extrasystoles were seen during intramyocardial cell injections in all cases, and no important ventricular arrhythmia was observed on computer-assisted Holter monitoring throughout 10–16 days of continuous ECG monitoring after the procedure in eight of the nine cases. One patient, not receiving prophylactic amiodarone, had episodes of VT and experienced two shots from his internal defibrillator at day 8 post-procedure.

Patients were discharged at days 10–16 post-transplantation and were well after 10 weeks of follow-up. Ambulatory Holter

Table 1 Summary of patients characteristics and procedural data

Patient number	Sex	Infarct location	Infarct duration	Procedure completed	Cell no. injected ($\times 10^6$)	Coronary vein cannulated	Injection volume (mL) per LV wall/area					LVEF (%)		NYHA class	
							Anterior	Inferior	Septal	Apical	Total	Baseline	6 months	Baseline	6 months
1	M	Anterior, septal	5 months	Yes	17	AIV	0.3		0.3		0.6	49	49	II	I
2	M	Inferior	5 years	Yes	35	MCV		0.2 + 0.2			0.4	38	45	II	I
3	M	Anterior, septal	6 years	Yes	68	AIV	0.5 + 1		1		2.5	31	35	III	I
4	M	Inferior	7 months	Yes	103	MCV		0.5		1	1.5	45	52	II	I
5	M	Anterior	5 years	Yes	49	AIV	0.5			0.5	1	43	47	II	I
6	M	Anterior	7 years	Yes	27	AIV	0.5 + 0.5				1	51	51	II	I
7	M	Apical, septal	8 years	Yes	80	MCV			0.25 + 0.5	0.25	1	43	48	III	I
8	F	Anterior, septal	6 months	Yes	32	AIV			0.5 + 0.25	0.25	1	30	35	III	I
9	M	Anterior	3 years	No[a]		N/A					N/A	33	33	II	II
10	M	Inferior, septal	8 years	Yes	106	MCV		0.3	0.5 + 0.3	0.5	1.6	35	35	III	I

AIV, anterior interventricular vein; MCV, middle cardiac vein; N/A, not applicable.
[a]Procedure abandoned because of inability to appropriately position the coronary sinus guiding wire across a valve present at the bifurcation of great cardiac vein.

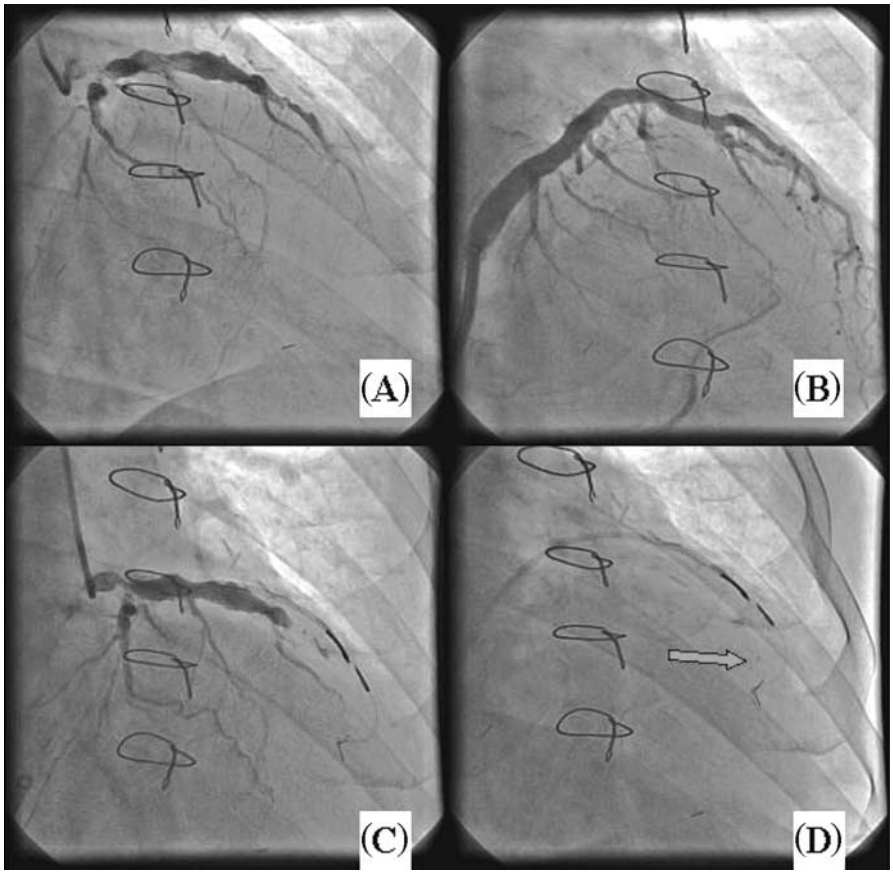

Figure 2 Procedural steps in patient 5: (*A*) coronary artery visualization in RAO 30 view; (*B*) administration of the contrast medium via a guiding catheter placed in the coronary sinus in RAO 30 view; (*C*) advancement of TrancAccess catheter system in the anterior interventricular vein and simultaneous visualization of the left coronary artery to assess the placement of the wire within the apex; and (*D*) advancement of the needle from the TransAccess catheter placed in the anterior interventricular vein and further advancement of the IntraLume microcatheter via the needle into the myocardium—the arrow indicates the microcatheter tip.

monitoring repeated every week during the follow-up period showed no episodes of sustained VT despite discontinuation of oral amiodarone 2–3 weeks after the procedure.

Secondary endpoint

Although efficacy evaluation was not the main purpose of the study, regular follow-up visits during 6 months follow-up were completed. After the procedure, the NYHA class improved in all nine patients and all subjects were in class I after 6 months follow-up. Ejection fraction evaluated independently by two blinded experienced investigators increased 3–8% in six out of nine cases, and no change in the ejection fraction, despite improvement in the NYHA class, in the remaining three patients was observed. Segmental contractility analysis was not statistically evaluated at this number of patients, but at least seven previously akinetic segments in the study population were hypokinetic 6 months after the procedure. None of the segments analysed deteriorated during 6 months follow-up.

Discussion

After introduction of myoblast transplantation during open chest surgery,[8,9,14] initial experience has been obtained with cell injection into myocardium with the use of endoventricular catheter systems.[11,12] We currently report procedural observations from a phase I clinical trial evaluating autologous myoblast transplantation performed with the use of a novel, percutaneous technique. Our study serves as both myoblast transplantation phase I trial and myocardial injection device evaluation study.

The use of the TransAccess catheter system allows direct myocardial access with IVUS guided needle punctures via the coronary venous system and cell infusion through a microcatheter into remote areas of injured myocardium. The system has been tested extensively on animal models[15] and may have potential advantages over the currently available approaches in the precise delivery of therapeutic agents into remote areas of myocardium. Although coronary sinus catheterization requires additional training even for an experienced interventionalist, it seems that IVUS guided needle injection (*Figure 4*) enables appropriate accuracy for intramyocardial advancement of microlumen injection catheter.

Currently available endoventricular catheter systems have limited stability, because the catheter does not follow the heart movements. In addition, the injection

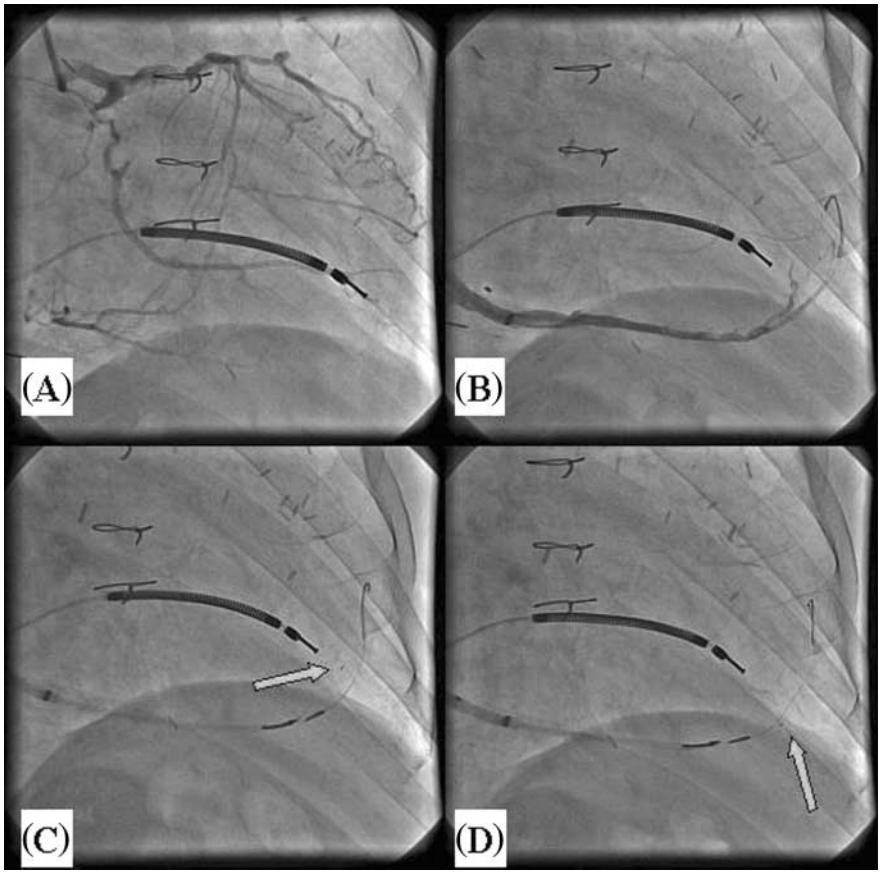

Figure 3 Procedural steps in patient 10: (*A*) coronary artery visualization in RAO 30 view; (*B*) the guiding catheter placed in the coronary sinus and oriented to middle cardiac vein with guide wire advanced along the vein; (*C*) and (*D*) advancement of the needle from the TransAccess catheter placed in the middle cardiac vein and further advancement of the IntraLume microcatheter via the needle into the myocardium with its tip (arrow) being placed in the apical area of the septum (*C*) and inferior wall (*D*).

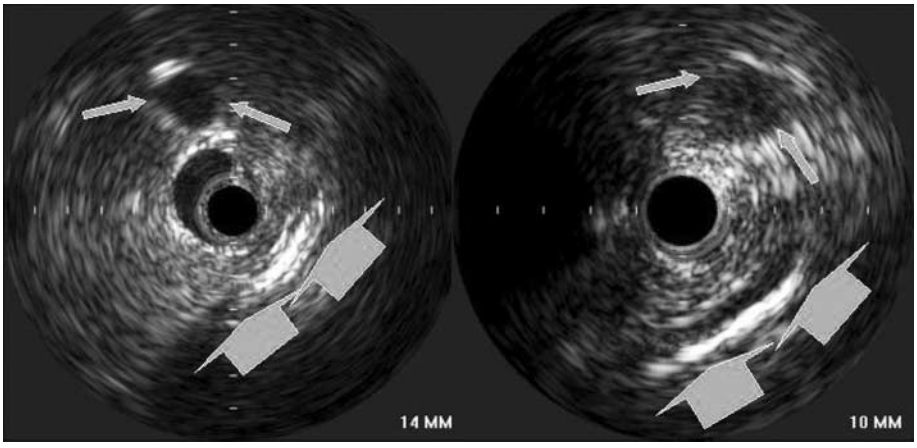

Figure 4 Intravascular ultrasound images obtained from the cardiac venous site. Please note the visibility of the pericardium (big arrows) and the coronary artery parallel to the vein (small arrows), enabling the orientation of TransAccess catheter system.

pressure with endoventricular systems can be destabilizing and cause expulsion of the needle tip from the injection site. In contrast, the TransAccess system moves with the heart wall (residing within the coronary vein) and allows for advancement of the microlumen injection catheter in a tangential direction with the myocardial tissue. In such a way, long tracks of cells may be deposited in the target area and less cell loss is anticipated with injections that are of a length measured in centimetres, rather than millimetres.

The results of our phase I trial show that the intramyocardial injections were possible to perform in nine out of

10 cases. The visibility of the coronary arteries parallel to the target coronary veins was good in all nine patients, allowing the safe intramyocardial needle injections with limited risk of artery puncture. Precise advancement of the microlumen catheter in the remote target area was evaluated under fluoroscopy using the coronary angiogram as a landmark for positioning the catheter tip within the injured myocardium (*Figures 2* and *3*). We believe that injections during the advancement of the microlumen catheter form a channel produced by the liquid pressure and, therefore, limit the myocardial trauma. As a result, no significant troponin release was observed, despite long, up to 4 cm deep injections, to deliver the cells to the target area. It should be noted that the use of the middle cardiac vein, parallel to the posterior descending coronary artery, in four cases resulted in the advancement of the TransAccess system closer to the apical segments of the left ventricle, when compared with the anterior interventricular vein. Lack of procedural success in one patient, related to the inability of appropriate positioning of the guiding catheter across the venous valve present at the bifurcation of the great cardiac vein, suggests the need for better coronary sinus guiding catheter design.

Experimental data as well as initial clinical experience have led to the initiation of clinical studies evaluating the use of totipotent stem cell or autologous skeletal myoblast transplantations as a possible means of myocardial replacement therapy[1] in patients with post-infarction myocardial injury. Both cell types have different properties, especially with respect to their different abilities to differentiate after transplantation under influence of surrounding tissue. Current data indicate that, for clinical application, bone marrow cells may be the best option when transplanted early after infarction, whereas autologous skeletal myoblast transplantation may be capable of restoring contractile performance within a fibrous post-infarction scar.[1] Because bone marrow derived stem cells do have the potential of extravasation into the injured tissue, clinical protocols evaluating the use of intracoronary administration of bone marrow stem cells have been applied[16] and are undergoing evaluation in several phase II/III trials. Skeletal myoblasts (satellite cells) do not extravasate under normal circulation pressure,[17] and therefore their potential application in myocardial regeneration requires cell injection into the area of myocardial injury.

In patients with chronic heart failure, the advantage of using skeletal myoblasts for cellular transplantation is their limited plasticity (i.e. possibility of differentiation into muscle fibres independently of environmental influence), their relatively good capacity to proliferate and grow in cell culture as well as high resistance to ischaemia.[1,18,19] The concept and its efficacy were evaluated in numerous studies on experimental animal models.[5,7,20–22]

Clear evidence is missing for formation of gap-junctions between transplanted myoblasts and cardiac myocytes. Gap-junctions being direct communication channels between the cytoplasm of cardiac myocytes are involved in electrical impulse travelling across the myocardium.[2,7] In addition, experimental data indicate that the beneficial effect of transplanted cells may result from strengthening the post-infarction scar and amelioration of left ventricular remodelling, independently of possible contraction effect.[23,24]

Initial clinical experience with autologous skeletal myoblast transplantation in a patient undergoing coronary artery bypass surgery has been reported by Menasché *et al*.[8] Five months later, they observed significant clinical status improvement including decrease of symptoms of heart failure by one NYHA class, increase in segmental contractility and ejection fraction seen on echocardiography, and increase in tracer activity on positron emission tomography suggesting new onset metabolic activity in the previously non-viable scar area. We have recently reported our independent phase I clinical trial on autologous skeletal myoblast transplantation in patients undergoing CABG.[9,10] An increase in segmental contractility was seen in all patients 6 months after the procedure, and this effect was maintained throughout a 12 month follow-up period.

Current experimental and clinical data suggest a possibility of increased risk of arrhythmogenicity in patients after autologous skeletal myoblast transplantation. In our CABG phase I experience,[9,10] episodes of sustained VT were observed in first two patients during early postoperative period, and this resulted in prophylactic administration of amiodarone in the other patients. Prophylactic amiodarone prevented VT episodes, and no amiodarone treatment was continued later than 6 weeks during follow-up. Because no significant ventricular arrhythmia despite no amiodarone treatment later during follow-up period was observed, we speculate that early arrhythmogenic effect of myoblast transplantation is rather related to intramyocardial injection, and/or inflammatory reaction against transplanted cells, especially against those dying shortly after injection, and is not necessary related to inappropriate electromechanical coupling due to the lack of gap-junctions formation between transplanted cells and surrounding cardiac myocytes (25).

On the basis of our previous experience with prevention of arrhythmia related to myoblast transplantation,[1,10] we decided to use prophylactic amiodarone infusion in our current series of percutaneous myoblast transplantations, with exception of patients having an ICD implanted. No episodes of VT after myoblast transplantation on prophylactic amiodarone, and VT episodes resulting in shots from his ICD in the only patient in this series not being on amiodarone suggests strongly an arrhythmogenic effect of myoblast transplantation and a preventive effect of amiodarone. However, the effect of amiodarone on electrophysiological properties of transplanted cells remains unknown. Potential relationship between the low number of cells injected and low incidence of arrhythmia could not be excluded. In contrast, at the current stage, having only small numbers of patients who have undergone autologous skeletal myoblast transplantations, it is difficult to predict whether skeletal myoblasts are really arrhythmogenic, especially when patients with ischaemic cardiomyopathy frequently do develop ventricular arrhythmia.[23,24] Nevertheless, future studies on cell transplantation in patients with post-infarction

heart failure will have to focus on potential arrhythmogenic effect.

During 6 months follow-up, we have observed improvement in NHYA class in all nine cases and increase ejection fraction in six out of nine patients. However, caution has to be taken, when interpreting phase I clinical trials results. Pronounced improvement in functional heart failure class and only limited increase in the ejection fraction suggest a strong placebo effect in our patients. The current paper reporting procedural phase I observations is just a description of procedural feasibility and safety of this novel technique of cell transplantation, and no final conclusions on efficacy should be drawn. We did not have minimum number of cells for this phase I trial, as it was believed that safety evaluation should be initiated with small number of cells. Definitely future phase II/III trials should have evaluated higher numbers of cells for efficacy evaluation. We believe, that our initial observations justify initiation of the phase II clinical trial to thoroughly assess safety issues and, subsequently, controlled efficacy clinical trials aiming at the evaluation of efficacy of percutaneous autologous myoblast transplantation in the treatment of post-infarction heart failure as well as to evaluate the TransAccess catheter system as a possible platform for future bioengineering therapies.

Acknowledgements

The assistance of Theodore Lamson, PhD, Ludwik Firek, MD, and Josh Makower, MD, all from TransVascular Inc., is gratefully appreciated. The work was supported in part by a governmental grant from Komitet Badań Naukowych and unrestricted research grant from Polpharma Foundation.

References

1. Siminiak T, Kurpisz M. Myocardial replacement therapy. *Circulation* 2003;**108**:1167–1171.
2. El Oakley RM, Ooi OC, Bongso A *et al*. Myocyte transplantation for myocardial repair: a few good cells can mend a broken heart. *Ann Thorac Surg* 2001;**71**:1724–1733.
3. Klug MG, Soonpaa MH, Koh GY *et al*. Genetically selected cardiomyocytes from differentiating embryonic stem cells form stable intracardiac grafts. *J Clin Invest* 1996;**98**:216–224.
4. Tomita S, Li RK, Weisel RD *et al*. Autologous transplantation of bone marrow cells improves damaged heart function. *Circulation* 1999; **100**:II247–II256.
5. Taylor DA, Atkins BZ, Hungspreugs P *et al*. Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. *Nat Med* 1998;**4**:929–933.
6. Li RK, Mickle DA, Weisel RD *et al*. In vivo survival and function of transplanted rat cardiomyocytes. *Circ Res* 1996;**78**:283–288.
7. Scorsin M, Hagège A, Vilguin J-T *et al*. Comparison of the effects of fetal cardiomyocyte and skeletal myoblast transplantation on postinfarction left ventricular function. *J Thorac Cardiovasc Surg* 2000;**119**:1169–1175.
8. Menasché P, Hagège AA, Scorsin M *et al*. Myoblast transplantation for heart failure. *Lancet* 2001;**357**:279–280.
9. Siminiak T, Kalawski R, Kurpisz M. Myoblast transplantation in the treatment of postinfarction myocardial contractility impairment. *Kardiol Pol* 2002;**56**:131–137.
10. Siminiak T, Kalawski R, Fiszer D *et al*. Transplantation of autologous skeletal myoblasts in the treatment of patients with postinfarction heart failure. *Circulation* 2002;**106**(Suppl. II): II-636.
11. Siminiak T, Fiszer D, Jerzykowska O *et al*. Percutaneous autologous myoblast transplantation in the treatment of postinfarction myocardial contractility impairment—report on two cases. *Kardiol Pol* 2003;**59**:492–496.
12. Smits PC, van Geuns RJ, Poldermans D *et al*. Catheter-based intramyocardial injection of autologous skeletal myoblasts as a primary treatment of ischemic heart failure. Clinical experience with six-month follow-up. *J Am Coll Cardiol* 2003;**42**:2063–2069.
13. Rozwadowska N, Fiszer D, Siminiak T *et al*. Evaluation of in vitro culture of human myoblasts for tissue autotransplants to the post-infarcted heart. *Kardiol Pol* 2002;**57**:233–240.
14. Herreros J, Prósper F, Perez A *et al*. Autologous intramyocardial injection of cultured skeletal muscle-derived stem cells in patients with non-acute myocardial infarction. *Eur Heart J* 2003;**24**:2012–2020.
15. Thompson CA, Nasser BA, Makower J *et al*. Percutaneous transvenous cellular cardiomyoplasty. A novel nonsurgical approach for myocardial cell transplantation. *J Am Coll Cardiol* 2003;**41**:1964–1971.
16. Strauer BE, Brehm M, Zeus T *et al*. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation* 2002;**106**:1913–1918.
17. Suzuki K, Murtuza B, Suzuki N *et al*. Intracoronary infusion of skeletal myoblasts improves cardiac function in doxorubicin-induced heart failure. *Circulation* 2001;**104**(Suppl. I):I213–I217.
18. Yaffe D, Saxel O. Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle. *Nature* 1977;**270**: 725–727.
19. Rozwadowska N, Fiszer D, Siminiak T *et al*. Evaluation of in vitro culture of human myoblasts for tissue autotransplants to the post-infarcted heart. *Kardiol Pol* 2002;**57**:233–240.
20. Atkins BZ, Leiws CW, Kraus WE *et al*. Intracardiac transplantation of skeletal myoblasts yields two populations of striated cells in situ. *Ann Thorac Surg* 1999;**67**:124–129.
21. Koh GY, Klug MG, Soonpaa MH *et al*. Differentiation and long term survival of C2C12 myoblast grafts in heart. *J Clin Invest* 1993; **92**:1548–1554.
22. Chiu RCJ, Zibaitis A, Kao RL. Cellular cardiomyoplasty: myocardial regeneration with satellite cell implantation. *Ann Thorac Surg* 1995;**60**:12–18.
23. Jain M, DerSimonian H, Brenner DA *et al*. Cell therapy attenuates deleterious ventricular remodeling and improves cardiac performance after myocardial infarction. *Circulation* 2001;**103**:1920–1927.
24. Sakai T, Li RK, Weisel RD *et al*. Autologous heart cell transplantation improves cardiac function after myocardial injury. *Ann Thorac Surg* 1999;**68**:2074–2081.
25. Menasché P, Hagège AA, Vilquin JT *et al*. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. *J Am Coll Cardiol* 2003;**41**:1078–1083.