Skeletal myoblast transplantation through a catheter-based coronary sinus approach: an effective means of improving function of infarcted myocardium

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Received 27 August 2004; revised 13 December 2004; accepted 23 December 2004; online publish-ahead-of-print 23 February 2005

KEYWORDS
Cell therapy; Skeletal myoblasts; Transplantation; Myocardial infarction; Percutaneous intervention

Aims This study was designed to assess the functional effects of a transvenous coronary sinus technique of skeletal myoblast delivery in infarcted myocardium.

Methods and results An anterior myocardial infarction was created percutaneously in 14 sheep. Simultaneously, a muscle biopsy was harvested and expanded. Two weeks later, sheep were instrumented percutaneously with a dedicated catheter incorporating an extendable needle for puncture of the venous wall and, under endovascular ultrasound guidance, a microcatheter was advanced through the needle into the target scar for cell delivery. Following the baseline echocardiographic assessment of left ventricular (LV) function, sheep were randomly allocated to receive four-staged in-scar injections of either autologous cells (n = 7) or culture medium (n = 7). Two months later, LV function was reassessed blindly and hearts were explanted for subsequent histological and immunohistochemical analysis. There were no acute procedural complications. Baseline LV ejection fraction (EF) was significantly lower in transplanted sheep than in controls [38% (35–48) vs. 51% (38–55), respectively, P = 0.03; median (range)]. Two months later, LV function was reassessed blindly and hearts were explanted for subsequent histological and immunohistochemical analysis. There were no acute procedural complications. Baseline LV ejection fraction (EF) was significantly lower in transplanted sheep than in controls [38% (35–48) vs. 51% (38–55), respectively, P = 0.03; median (range)]. Clusters of myoblasts were identified by histology and immunohistochemistry in three of the seven transplanted sheep.

Conclusion These data suggest the functional efficacy of the transvenous coronary sinus technique as a less invasive means of cell delivery to infarcted myocardium.

Introduction

In the setting of severe heart failure, cellular transplantation has recently emerged as a potentially new treatment option for enhancing function of extensively infarcted myocardium.1 Although the optimal cell type remains to be determined, we and others have more specifically assessed the effects of surgically implanted skeletal myoblasts and have shown their ability to improve regional and global left ventricular (LV) function in experimental infarction models.2–6 Early clinical trials have confirmed the feasibility of myoblast transplantation in combination with coronary artery bypass grafting7,8 and a randomized study is currently underway to assess efficacy of the procedure.

However, the invasiveness of the surgical approach, particularly in those high-risk patients who do not require concomitant revascularization, has justified the search for an alternate percutaneous mode of cell delivery. Both endovascular and coronary sinus transvenous catheters have thus been developed to meet this objective. We have decided to explore the latter technique because it features some distinct advantages: it is relatively simple to implement, provides a stable and direct access to all areas of the heart, and allows an accurate delivery of cells directly into the core of the target scar. The present experiments were thus designed with the dual objective of (i) assessing the ability of this route to achieve a successful engraftment of...
skeletal myoblasts into the targeted areas and (ii) validating its functional efficacy in a large animal model of myocardial infarction.

Methods

This investigation conforms to the 1996 Guide for the Care and Use of Laboratory Animals and with the Animal Welfare Act.

Myocardial infarction model

Sheep weighing 45–50 kg were anaesthetized (thiopental sodium, 15 mg/kg) and tracheally ventilated. After selective catheterization of the left coronary artery using the Seldinger technique and opacification with ioxithalamate sodium (Guerbet, Aulnay/Bois, France), an anterior myocardial infarction was created by the release of a thrombogenic coil (Cook, Bloomington, IN, USA) in the middle part of the left anterior descending coronary artery under fluoroscopic guidance. Care was taken to capture the venous follow-through phases to picture coronary venous anatomy.

Cell cultures

At the time of infarction, a muscle biopsy was obtained from the vastus lateralis and processed for further expansion as previously described. At the completion of the 2 week up-scaling phase, cells were harvested by trypsination, washed three times, and concentrated in saline 0.9% supplemented with bovine serum albumin (0.5%, Sigma, St Louis, MO, USA). Aliquots were retained for assessment of the proportion of CD56-positive myogenic cells and viability using flow cytometry and propidium iodide exclusion, respectively. Cells were then kept on ice for transportation and warmed at room temperature immediately before injections.

Experimental groups

In preliminary in vitro experiments, the effect of passage through the microcatheter (TransVascular, Inc., Menlo Park, CA, USA) was assessed by injecting the cells proximally and recovering them distally for measurement of viability. In vivo experiments were then performed in the sheep model of percutaneously induced myocardial infarction. Fourteen sheep survived the procedure. Two weeks after creation of infarction, they were randomized to receive either autologous myoblasts grown from the biopsy taken 2 weeks after the procedure. Fourteen sheep survived the procedure. Two weeks after creation of infarction, they were randomized to receive either autologous myoblasts grown from the biopsy taken 2 weeks earlier (n = 7, transplanted group) or an equivalent volume of culture medium (n = 7, control group). The number of animals was based on a previous study showing that such a sample size should allow the demonstration of a significant difference between groups, if any.

Cell transplantation

Under general anaesthesia, animals were percutaneously instrumented with a 9F venous femoral sheath. The coronary sinus was accessed with a 7F Porcine 3 catheter. A 260 cm, 0.035 in. hydrophilic-angled wire (Terumo, Tokyo, Japan) was advanced into the anterior interventricular coronary vein through the great cardiac vein. The 7F catheter was then withdrawn, keeping the wire in place, and a 9F CS guiding catheter and dilator (TransVascular) were placed with conventional over-the-wire technique. The hydrophilic guide wire was then exchanged for a 0.014 in. guide wire and the dilator was removed. The TransAccess catheter (TransVascular) which incorporates a tipped phase-array ultrasound probe for guidance and an extendable 24-gauge nitinol needle for puncture of the venous wall was advanced over the 0.014 in. guide wire to the distal part of the anterior interventricular coronary vein for myocardial access. Intravascular orientation before extending the needle was appreciated under intravascular ultrasound imaging and a 27-gauge microinfusion catheter (IntraLume, TransVascular) was then advanced through the needle into the myocardium under fluoroscopic guidance. Each animal received four-staged in-scar injections of autologous cells or an equivalent volume of culture medium also supplemented with the same concentration of bovine serum albumin. All infusions were performed under intravascular ultrasound guidance (Volcano Therapeutics, Rancho Cordova, CA, USA). The tactile feeling, when moving forward the micro-catheter, provided additional evidence that the injections were made in a fibrotic area. Preliminary evaluations were performed to confirm the adequate infusion into the fibrotic scar using a blue dye.

Assessment of results

Function

LV function was assessed by transthoracic two-dimensional echocardiography using a 5.0 MHz probe (5V2c) connected to a Sequoia 512 system (Acuson, Mountain View, CA, USA). Examinations were performed immediately before injections and 2 months thereafter in the 14 surviving sheep. One parasternal LV long-axis view was standardized to include the mitral and aortic valves, and the apex and two parasternal short-axis views were standardized to include the two papillary muscle tips (papillary muscle short-axis view) and the apex (apex short-axis view) at the mid level between the papillary muscle short-axis view and the true LV apex. These views were digitally stored online and subsequently re-examined offline in a blinded fashion for measurements at end-diastole and end-systole of (i) the LV long-axis dimensions (L) on the parasternal long-axis view, as the distance between the middle of the axis connecting the upper aortic and lower mitral annular hinge points and the apex; (ii) the LV short-axis area (A1) at the papillary muscle level; (iii) the LV short-axis area (A2) at the apex level. This enabled the calculation of end-diastolic (EDV) and end-systolic (EDS) LV volumes using the cylinder truncated cone–cone simplified formula

$$A_1 \left( \frac{L}{2} \right) + \left( A_1 + A_2 \right) \left( \frac{L}{6} \right) + A_2 \left( \frac{L}{9} \right)$$

in order to calculate LV ejection fraction (LVEF, %)

\[
\frac{EDV - EDS}{EDV} \times 100
\]

Each measurement was a mean of three consecutive cycles. To assess regional function, scar thickness was measured, after the left ventricle has been divided into 16 segments, and the kinetics of the four myocardial segments most involved by the infarction were graded on a 0–3 scale (0: normal; 1: hypokinesia; 2: akinesia; 3: dyskinesia); a regional function index was then calculated by dividing the sum of the individual scores by the number of segments. The four segments were adjusted according to the size and location of the infarct.

Pathology

After the last echocardiographic assessment, the animals were sacrificed. The hearts were removed from the chest and fixed in formalin. The dissection of the heart was performed according to the following sequence: after opening the right appendage and identification of the ostium of the coronary sinus, the coronary sinus and the great vein were cut longitudinally; the posterior left main vein was then serially sectioned with a blade up to the apex; any thrombus in the veins as well as the position of the coil in the adjacent coronary artery were assessed; the heart was then cut in 5 mm thick serial biventricular sections from apex to basis; finally, the infarcted area was assessed for its extent, location, and transmurality. The whole infarcted area including the posterior left main vein, and the coronary sinus and the great vein were embedded in paraffin (range: 12–18 paraffin blocks per sheep). The sections were stained with haematoxylin and eosin (H&E) and assessed for the presence of myotubes, inflammation, thrombosis, or lesions of the venous system. Myocardial sections in the area of the myoblast...
injections were immunostained with an antibody against the skeletal muscle fast myosin heavy chain (MHC) isoform (clone My32, Sigma) to validate the data obtained by plain H&E stain.\textsuperscript{11}

**Statistics**

Statistical analyses were performed using the Statview 5.0 software (SAS Institute, Cary, NC, USA). The non-parametric two-sided Mann–Whitney U-test was used for comparing data between groups with the Holm’s correction for multiple testing. Consequently, results are reported as median (minimal–maximal) values. Statistical significance of \(P\)-value was set at \(<0.05\).

**Results**

**Cell cultures**

Cultures yielded an average of 240.7 million cells (range: 100–575), of which 64\% (range: 36–95\%) were CD56-positive. After passage through the micro-infusion catheter, their viability was not altered and still averaged 88.6\%.

**Efficacy data**

**Functional assessment**

Myocardial infarction was created in 34 animals. Twenty animals were excluded because of lethal ventricular fibrillation after myocardial infarction (\(n = 19\)) and an infectious pneumopathy after transplantation (\(n = 1\)). This let a study group of 14 animals equally distributed among the control and myoblast groups (Table 1). In spite of randomization, baseline mean L VEF turned out to be lower in transplanted sheep [38\% (35;48)] than in controls [51\% (38;55), \(P = 0.03\)]. However, 2 months later, L VEF had significantly deteriorated in control sheep [39\% (36;47)], whereas it significantly improved in myoblast-injected hearts [50\% (47;56), \(P = 0.002\) vs. controls] (Figure 1). When expressed as a percentage relative to baseline EF, these figures translate into a 13\% (−35;0) decrease in EF in controls vs. a 33\% (4;54) increase in treated hearts (\(P = 0.002\) vs. controls). In contrast with global function data, the regional function index, encompassing the most infarct-involved segments, did not significantly differ between the two groups immediately before myoblast injections [controls: 2.25 (1;3); cell-transplanted: 1.25 (1;2), \(P = 0.14\)]. The patterns of changes were then markedly different between the two groups as this index remained unchanged in control hearts [1.75 (0.25;3)], whereas it decreased after myoblast transfer to 0.5 (0;1.25), \(P = 0.03\) vs. controls. The benefits of myoblast transplantation were primarily related to a significantly smaller increase in end-systolic LV volumes [0\% (−29; +35) vs. 48\% (6;86) in controls, \(P = 0.018\)], whereas the increase in LV end-diastolic volumes did not differ between the two groups [18\% (−3; +68) and 13\% (3;60) in control and transplanted hearts, respectively, \(P = 0.6\)]. Likewise, scar thickness (cm) did not differ significantly between the two groups, either at baseline [controls: 0.59 (0.45;0.64); cell-transplanted: 0.49 (0.43;0.62), \(P = 0.3\) or 2 months after transplantation [controls: 0.59 (0.50;0.62); cell-transplanted: 0.57 (0.54;0.68), \(P > 0.9\)]. There were no

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**Figure 1** Changes in left ventricular ejection fraction (EF) before and after transplantation (Tx).
acute or delayed post-injection complications like pericardial blood effusion or arrhythmias.

**Histological assessment**

In all sheep, a transmural infarction involving the apex, anterior septum, and posterior wall was identified, which is consistent with the distribution of the sheep left anterior descending artery which extends to the inferior aspect of the LV. However, grafted myotubes embedded in scar tissue could only be detected in three transplanted hearts. They were present in several spots adjacent to the vein, mainly located in the subepicardial layers of the heart, and featured multinucleated structures with highly organized myofilaments including Z lines. Grafted myotubes were lying parallel to fibrous tissue without evidence for intercellular connections. Neither inflammation nor patterns of an immune reaction that might have been triggered by bovine serum albumin present in the injection medium (with or without cells) were observed in any specimen (Figure 2, see Supplementary material online). Likewise, there was no evidence for myocardial perforation, pericardial effusion, or coronary vein obstructive thrombosis. However, the procedure induced a non-obstructive mural thrombus in the coronary sinus and the great vein in four cases (one control and three cell-treated). Two of these animals had an additional non-obstructive thrombosis of the posterior left main vein. No vein wall damage or rupture were otherwise observed. Likewise, the procedure did not induce any haemorrhagic or inflammatory changes within the infarcted scar.

**Discussion**

The major finding of the present study is that the transvenous coronary sinus approach allows delivery of skeletal myoblasts into post-infarction myocardial areas, and that their engraftment is of sufficient magnitude to elicit a significant improvement of global LV function which is reflected at the regional level of the infarcted segments by a lower wall motion score index compared with the control medium-injected group.

**Advantages of the transvenous coronary sinus approach**

In the setting of less invasive procedures for delivering cells to infarcted myocardium, the coronary sinus approach features distinct advantages: (i) technically, it is relatively straightforward and can be completed expeditiously; the ‘tips and tricks’ gained from experience with cardiac resynchronization therapy may further help to make it user-friendly; (ii) this route of administration is also spatially versatile in that the coronary venous system acts as a roadmap from which catheterization of both the anterior and posterior interventricular cardiac veins gives access to all areas of the heart, including the interventricular septum; (iii) the endovascular ultrasound guidance of the catheter provides a safe and accurate means of targeting areas to be injected; the tip of the catheter then behaves like a drill that tunnels through myocardial tissue and allows widespread cell delivery; and (iv) the coaxiality of the injection microcatheter relative to the surrounding myocardial tissue allows it to move with heartbeats so that the problem of effective maintenance of catheter contact with the moving ventricular wall of a contracting heart, which is inherent in the endoventricular approach, is irrelevant to the transvenous technique; furthermore, the deep penetration of the infusion microcatheter into the core of the target area only occurs through a limited number of venous punctures (on average, three to four per injected area in this study).
Along with the stability of the catheter, this should reduce back leakage along the needle tract and thus optimize cell retention in the areas to be grafted.

**Previous studies**

In spite of these presumed advantages, pre-clinical data on cell transfer by the coronary sinus transvenous technique are still scarce and in fact primarily limited to the study of Thomson et al., who have investigated bone marrow cell delivery in a pig model on non-infarcted myocardium.

The clinical experience with this approach is still equally limited. There was oral reporting of some patients receiving skeletal myoblasts transvenously in the Thoraxcenter at Rotterdam, but the most important series (10 patients) is probably the Poznan trial conducted by Siminiak et al. Overall, these data have primarily established the technical feasibility (there was only one failure due to the inability to catheterize the coronary sinus in Siminiak’s series) and the safety (no adverse events have yet been reported) of the procedure, but they failed to provide evidence that this route of cell delivery allowed an engraftment of sufficient magnitude to improve function. The present study was precisely designed to fill this gap.

**Interpretation of results**

The present results are, overall, supportive of the safety and efficacy of the transvenous approach. There were no procedural complications except for non-obstructive venous mural thrombosis in several cases. However, the catheter used in the present study has been designed for the human coronary venous system which markedly differs from that of sheep. Consequently, successful catheterization of the target veins often required repeated attempts and we speculate that this resulted in venous endothelial damage which, despite adequate anticoagulation, set the stage for thrombosis. On the basis of the current experience with cardiac resynchronization therapy, this should not be a clinically relevant concern. Furthermore, although the animals were not monitored by Holter recordings during the period of follow-up, none of them experienced sudden death or any other complication suggestive of severe arrhythmic events. Evidence for efficacy is based on the observation that, although myoblast-treated sheep had initially lower EFs than the controls, they actually improved their function over time after transvenous cell delivery so that, at the 2 month post-transplantation study point, their EFs were significantly higher than in control medium-injected sheep. Regional function of the infarcted segments featured a similar pattern with a decrease in the regional score index (indicating better kinetics) in the myoblast group while this parameter remained unchanged from baseline values in controls. Analysis of LV volumes suggests that the transplantation-related improvement was primarily due to a better preservation of systolic function as remodelling did not differ between the two groups.

A puzzling observation, however, is that although clusters of skeletal myoblasts were consistently identified in a pilot study entailing skeletal myoblast transplantation in four non-infarcted hearts (data not shown), grafted cells were only found in three out of the seven infarcted hearts that were transplanted. With the limitation inherent in the small sample size, there was no apparent correlation between the number of injected cells and their subsequent identification. Indeed, it is now well documented that a large proportion of injected cells die shortly after epicardial or endocardial delivery and although the mechanism of cell death is multifactorial, hypovascularization of the target area is likely to be important, as suggested by the enhancement of graft survival by interventions that promote angiogenesis. This hypothesis is further supported by the present findings of a discrepancy in the extent of myoblast engraftment between normally perfused myocardial tissue (four successful engraftments in the four hearts of the pilot experiments) and infarcted scars (only three out of seven). Although the current study was not designed to provide mechanistic insights, our findings that the lack of detectable cells did not preclude LV systolic function to improve along with the well-established inability of grafted myoblasts to couple with host cardiomyocytes to form a functional syncitium rather lend support to the paracrine hypothesis whereby myoblast-released cytokines and or growth factors could favourably affect extracellular matrix composition, rescue hibernating cardiomyocytes or even recruit resident cardiac stem cells.

These beneficial effects might then perpetuate over time even when the source cells have died, which massively occurs during the early post-implantation period and tends to level off thereafter.

**Study limitations**

Several limitations of this study need to be acknowledged. The first pertains to the finding that even though hearts in which myotubes could not be found demonstrated an improved LV function, this lack of engraftment raises the possibility that the technique may not be consistently efficacious in ensuring cell delivery. In future, improvements in catheter design and guidance systems should hopefully allow to fix this problem. Secondly this study did not specifically assess the potential interactions between cells and the catheter material and, more specifically, the effects of lumen-coating lubricants and infusion pressures on myoblast proliferation and/or differentiation. Thirdly, the variability of the coronary venous anatomy in the human heart may complicate the procedure or even make it impossible under safe conditions. Likewise, technical difficulties contra-indicating even an attempt to catheterize the coronary sinus can be expected in patients having undergone prior cardiac resynchronization therapy, which is an increasingly common situation in the heart failure population. Finally, the results could only be assessed in the select group of animals that survived the coronary artery embolization procedure and, as such, they may not be representative of the myocardial infarction population at large.

**Conclusions**

In spite of these limitations, this study shows that the coronary transvenous sinus technique may afford an engraftment of skeletal myoblasts into infarcted areas and an associated improvement in LV function. Irrespective of the place it may occupy in the armamentarium of cell delivery techniques, its encouraging performance still requires further validation focusing particularly on the optimization of cell engraftment and cell–material interactions.
Supplementary material

Supplementary material is available at European Heart Journal online.

References
