Heart failure is a major and expanding public health concern; in most cases, it results from myocardial infarction, leading to an akinetic fibrous scar and associated left ventricular (LV) remodeling. Despite dramatic improvements in the medico-surgical management of these patients, their persistently dismal outlook (1) still justifies the ongoing search for new treatments.

In this setting, cellular transplantation is receiving a growing interest as a means of replacing lost cardiomyocytes by exogenously supplied contractile cells, among which the skeletal myoblasts, which are responsible for skeletal muscle formation and repair, feature clinically attractive characteristics, including ease of procurement, growth potential in vitro, and strong resistance to ischemia.

Experimentally, engrafted skeletal myoblasts differentiate into myotubes and improve postinfarction cardiac function (2–8). These observations have set the stage for this phase I human trial in which the first patient was operated on June 15, 2000 (9).

**METHODS**

**Inclusion criteria.** Eligibility for inclusion was based on: 1) systolic LV dysfunction, as reflected by an echocardiographic LV ejection fraction (LVEF) ≤ 35%; 2) a history of myocardial infarction with a residual akinetic and nonviable scar, as demonstrated by a lack of response to low-dose...
Global echocardiography was performed using fundamental and preoperative screening. Giving their witnessed consent, patients were included after having received extensive information and Health Authorities and our ethics committee approval. Patients were excluded from the transplanted area and viable but ischemic myocardium. The main exclusion criteria consisted of diabetes, coronary artery bypass graft (CABG) in remote (i.e., different from the transplanted area) and viable but ischemic myocardium. The main exclusion criteria consisted of diabetes, cardiacogenic shock, unstable angina requiring urgent surgery, peripheral muscular dystrophy, and positive serologic test results for human immunodeficiency virus and hepatitis.

This protocol was approved by the French Regulatory Health Authorities and our ethics committee. Patients were included after having received extensive information and giving their witnessed consent.

Preoperative screening. Transthoracic two-dimensional echocardiography was performed using fundamental and second-harmonic imaging. Global LVEF (%) was calculated from measurements of LV end-diastolic volume (LVEDV) and LV end-systolic volume (LVESV; both in ml) using the biplane Simpson’s rule, as \( \frac{LVEDV - LVESV}{LVEDV} \times 100 \) (10). Regional contractile function was assessed semiquantitatively after division of the LV into 16 segments, as recommended by the American Society of Echocardiography (10). Care was taken to optimize endocardial visualization on three parasternal short-axis views of the LV (at the base, mid-ventricular level, and apex) and apical two-, three-, and four-chamber views. Segmental thickening of each segment was then visually evaluated and graded as dyskinetic, akinetic, severely hypokinetic, moderately hypokinetic, or normal (11). This initial assessment was performed within the month before surgery, not during episodes of cardiac failure, and at rest; and dobutamine (5, 10, and 15 μg/kg per min without exceeding a 10-beats/min increase in heart rate) was used to check for the absence of induced segmental thickening in the infarcted area (12,13).

The \(^{18}\text{FDG}\) PET studies were performed with a whole-body scanner (ECAT Exact, CTI/Siemens, Knoxville, Tennessee) with an intrinsic spatial resolution of 6 mm full width at half maximum. To augment myocardial glucose uptake, each patient received 50 g of glucose orally. One hour later, \(^{18}\text{FDG}\) (370 MBq) was injected intravenously and six images of 5 min each were acquired after 13 min. The relative regional myocardial \(^{18}\text{FDG}\) uptake was determined on three short-axis slices that corresponded in location to the echocardiographic sections and to the surgical map depicting the sites of cell injections. Regional myocardial \(^{18}\text{FDG}\) activity concentrations were defined as the ratio over the maximum myocardial \(^{18}\text{FDG}\) uptake. Segments with \(\leq 50\%\) \(^{18}\text{FDG}\) uptake were defined as nonviable (14).

Staging of the procedure. Muscular biopsy. A 10- to 15-g piece of the vastus lateralis muscle was retrieved from the patient’s thigh under local anesthesia, minced, and transported in a preservation medium to the Cell Therapy Laboratory.

**CELL CULTURES.** The general methodology has been previously described (9). Briefly, the muscle biopsy was digested using collagenase and trypsin. The primary cell culture was expanded in cell factories using a clinical grade myogenic-specific medium based on a modification of MCDB120, supplemented with bovine fetal serum, basic fibroblast growth factor, dexamethasone, penicillin, and streptomycin. The cells were expanded and harvested before reaching confluence by trypsinization. Scale-up was performed to achieve the following targets: at least 500 \(\times 10^6\) cells, \(\geq 50\%\) myoblasts, and \(\geq 70\%\) viable cells. Finally, cells were collected by trypsinization, washed, and suspended in albumin (150 \(\times 10^9\) cells/ml). Quality control was performed throughout the process and for the final product. The percentage of myoblasts and viable cells was quantified by flow cytometry after staining for CD56, a marker specific for skeletal muscle cells, and upon exclusion of propidium iodide, respectively. Preclinical double-labeling fluorocytometric analyses have demonstrated that most CD56-positive cells were also positive for desmin. Short- and long-term microbiologic control studies were performed repeatedly. The cell-containing suspension was transferred into sterile syringes, which were shipped back at room temperature to the cardiac surgery department. One aliquot of the final product was retained for testing the myogenic capacity of the cells, and, in the first five patients, another aliquot was used for tumorigenic testing by injecting 5 \(\times 10^5\) cells intravenously into irradiated nonobese diabetic-severe combined immunodeficient mice.

**CELL TRANSPLANTATION.** Under cardioplegic arrest, the cell suspension was injected within and around the scar, using a customized prebent 27-gauge needle, allowing tangential injections and thus reducing the risk of intracavitary cell delivery.

There was no specific postoperative treatment except for corticosteroids (prednisolone, 780 mg), given over the first two postoperative days.

**Assessment of outcomes.** The primary end points were feasibility and safety. Feasibility was defined as the ability of the expansion procedure to yield the target numbers of cells within two to three weeks. Safety referred to adverse events related to biopsy/cell reimplantation and specifically involved detection of ventricular arrhythmias by 24-h Holter.
electrocardiographic (ECG) monitoring studies performed before and within the first three postoperative months.

The secondary end point was efficacy, which was primarily assessed by two-dimensional echocardiographic analysis of LV function at one, three, and six months postoperatively and at the end of follow-up. In addition to measurements of global LVEF, serial changes in LV segmental thickening were evaluated semiquantitatively and classified as unchanged, improved (≥1 grade), or worsened (≥1 grade).

These grafted areas were localized by reference to the intraoperative diagram depicting the injection sites. Image analysis was performed by two investigators, one of whom was blinded to the timing of echocardiography, the number and location of coronary bypasses, and the sites of cell injections.

Statistics. Results are reported as the mean value ± SEM. A comparison of preoperative and postoperative data was done using the paired t test, with p < 0.05 as the limit of significance.

RESULTS

Study group. The study group (Table 1) comprised 10 male patients with a mean age of 60 ± 3 years (range 38 to 73). The age of the infarct was 6.2 ± 1.9 years (range 1 month to 19 years), and its location was anterior (n = 6), posterior (n = 3), or posterolateral (n = 1). All of the infarcted areas were angiographically subserved by totally occluded, nonrevascularizable coronary arteries.

All patients had evidence of myocardial ischemia, as demonstrated by angina or stress tests, and/or clinical symptoms of congestive heart failure. Four patients were in New York Heart Association (NYHA) functional class II; five in class III; and one in class IV. The LVEF was 24.3 ± 1.3% (range 18% to 34%), and the 18FDG uptake ratio was 0.41 ± 0.04.

Cell cultures. Histologic analysis of the biopsied muscle revealed normal anatomy. The target numbers of cells were consistently achieved in all patients within two to three weeks (i.e., 953 ± 71 × 10⁶ cells [range 530 to 1,215] were produced in 16 ± 1 days [range 14 to 20]). All cultures contained more than 60% myogenic cells (mean 86 ± 3% [range 67% to 97%]) and more than 90% viable cells (mean 95 ± 1% [range 86% to 99%]). There was no bacterial or fungal contamination. None of the NOD-SCID mice injected with myoblasts developed a macroscopically detectable tumor. Myoblasts that were replated at the time of cell collection grew to confluence and then differentiated and fused to form multinucleated myotubes characteristic of skeletal muscle tissue.

Cell transplantation. All patients had two bypass grafts except for one patient who had three. An average volume of 5.7 ± 0.3 ml (range 4 to 8) containing 871 ± 62 × 10⁶ (range 500 to 1,115) was injected in 37 ± 3 sites (range 27 to 57) throughout the scar area (29 ± 3 cm² [range 20 to 40]). The procedure averaged 18 ± 1 min (range 15 to 26) and was consistently uneventful.

Early postoperative course. There was no complication related to the muscular biopsy. One patient (no. 5) in NYHA class IV with a history of three previous infarctions died early after the operation, likely due to a mesenteric infarction subsequent to a profound hemodynamic deterioration, which occurred suddenly before cardiopulmonary bypass was instituted. The postoperative course of the other nine patients was uneventful.

Short-term outcomes. By April 1, 2002, the follow-up period averaged 10.9 ± 4.5 months (range 5 to 17.5).

The only adverse event likely related to the procedure was the occurrence of sustained, monomorphic ventricular tachycardia (VT) featuring a right conduction delay pattern in four patients, three of whom had preoperative ventricular hyperexcitability (grade 2 to 3 in Lown’s classification). All these VTs occurred early after the operation (11 to 22 days) and, except for one syncopal form, were clinically well tolerated. None of these arrhythmias occurred in the setting
of myocardial ischemia, which was consistently ruled out by the absence of suggestive clinical and ECG changes and full angiographic patency of all grafts. The four patients were implanted with an automatic internal cardioverter-defibrillator (AICD), either shortly after the operation because of the inducibility of VT on programmed ventricular stimulation (n = 2; Patient nos. 7 and 8) or later (Patient nos. 4 and 6) because of a delayed recurrence of VT (at five and nine months postoperatively), despite antiarrhythmic drug therapy (beta-blockers and amiodarone). Among the two patients who had late device implantation, only one had undergone an early postoperative stimulation, which failed to induce VT. At follow-up after AICD implantation (one month in one case and eight months in the other three cases), only one of the four device-implanted patients had experienced two appropriate shocks. In the last three patients in the trial, amiodarone therapy was prophylactically instituted at the time of biopsy and continued for three months. One patient still experienced asymptomatic VT (he is one of those who received an early postoperative defibrillator), but at the time of the latest follow-up visit, the two others had remained arrhythmia-free. The small size of the sample did not allow us to conclusively establish a correlation between the number of injected cells and the occurrence of VT.

Efficacy data pertain to eight of the nine operative survivors, as the poor echogenicity of Patient no. 7 led to his exclusion from further segmental analysis by the blinded observer. For these eight patients, there was complete agreement between the two observers regarding the postoperative improvement in systolic thickening ≥1 grade. In this cohort, LVEF rose from 23.8 ± 3.9% (range 18% to 31%) preoperatively to 32.1 ± 7.5% (range 25% to 45%) postoperatively (p < 0.02), whereas LVEDV remained statistically unchanged (124 ± 46 ml/m² [range 56 to 154]) postoperatively vs. 107 ± 31 ml/m² [range 69 to 198] preoperatively). According to the surgical map, 22 in-scar segments were implanted with cells. Postoperatively, 14 of them (63%) demonstrated new-onset echocardiographic systolic shortening (6 of 8 patients), with the number of improved segments increasing over time (from 8 before 3 months to 14 at the end of follow-up). In comparison, 26 noninjected but revascularized segments improved echocardiographically to a similar extent as the grafted ones after the procedure. This amelioration translated into an improvement in NYHA class from 2.7 ± 0.2 to 1.6 ± 0.1 (p < 0.0001), whereas preoperative medications were continued unchanged after surgery, except for diuretics, the dose of which was tailored to the magnitude of congestive symptoms. Only one patient required a brief rehospitalization for a transient episode of heart failure related to inadvertent discontinuation of diuretics. One patient (Patient no. 1) died of a stroke at 17.5 months postoperatively. The improvement of his cardiac function (both global and segmental), which was manifest early postoperatively, was sustained thereafter until the time of his death. Autopsy found clusters of myotubes embedded in the scar tissue, without evidence of cardiomyogenic differentiation, as reflected by the lack of gap junction formation.

**DISCUSSION**

**Feasibility.** Our first objective was to assess whether cell culture procedures routinely used experimentally could be clinically upgraded so as to make them compatible with human use. From this standpoint, the present results are satisfactory, as a large number of cells could reproducibly be grown from the initial muscle biopsy within two to three weeks and in strict compliance with Good Manufacturing Practice constraints. Because the functional outcome following myoblast transplantation is related to the number of injected cells (15), the lower target cell yield was set at 500 × 10⁶, at least 50% of which were required to be myoblasts. Our data show that these thresholds were consistently overshot. Although it is technically possible to grow more than one billion cells, such numbers would require larger injection volumes, which raises a potential safety issue. Reducing this volume by increasing the cell concentration would, in turn, expose the cells to damage subsequent to their passage through a necessarily small-bore needle.

Cultures were obtained expeditiously in all patients, regardless of their age and comorbidities. Likewise, there was little variability in the percentage of myogenic cells or their functional capacity to form multinucleated myotubes in vitro.

**Safety.** There were no perioperative complications related to biopsy or cell injections. The two early and late deaths were clearly unrelated to the cell transplantation procedure.

The absence of tumor in myoblast-injected immunodeficient mice confirms that oncogenicity is unlikely to be a concern with autologous transplantation of primarily cultured skeletal myoblasts. When these myoblasts were exposed to the differentiation medium, they never turned to cells of a different nature (i.e., osteoblasts, chondroblasts). This commitment to a strictly myogenic lineage is due to the already advanced stage of differentiation of myoblasts and contrasts with the greater phenotypic plasticity of bone marrow stem cells, which are also considered for intramyocardial grafting.

The only serious adverse event likely related to the procedure was the occurrence of ventricular arrhythmias in four patients, although such a relationship may be confounded by the trend for patients with ischemic cardiomyopathies to develop arrhythmic events (16). Skeletal myoblasts and cardiomyocytes express different sets of ion channels (17) whose distinct kinetic activation/inactivation patterns could result in arrhythmogenic inhomogeneities in action potential conduction. In fact, inhomogeneous intramyocardial distribution of gap junctions increases the risk of arrhythmias (18). Alternatively, these arrhythmias could have an inflammatory origin due to both needle punctures and early cell death from necrosis and/or apoptosis, as...
shown in other models of cell transplantation (19,20). These events would then trigger the release of inflammatory cell-derived arrhythmogenic products, as occurs during myocarditis (21). Finally, reentry pathways could be created by cell-independent, injection-related disorganization of the supracellular myocardial anatomy in the border zone. These various hypotheses are currently under experimental investigation.

In the four patients who experienced VT, the decision to implant a defibrillator was based on standard transplantation-independent criteria and is consistent with the survival benefit expected from the device in this patient population at high risk of severe arrhythmic events, as shown by the recently reported Multicenter Automatic Defibrillator Implantation Trial (MADIT) II trial (22).

**Efficacy.** This phase I trial was neither designed nor powered to assess the efficacy of the procedure, so only careful and preliminary conclusions can be drawn from the comparison of postoperative versus preoperative data.

As improvement in symptoms and LVEF may simply reflect efficacious revascularization, analysis of echocardiograms primarily focused on contractile changes of the cell-transplanted scarred segments. Fourteen of 22 of these segments had improved kinetics, as evidenced by new-onset systolic thickening. Experimentally, myoblast transplantation limits postinfarction ventricular dilation (23), but this mechanism was probably not operative in our patients with old infarctions and already enlarged ventricular dimensions that did not regress postoperatively. Echocardiographic data rather suggest a contribution of engrafted myotubes to contractile function. As these cells do not express connexin 43–supported gap junctions with host cardiomyocytes (24), classic electromechanical coupling is unlikely. However, skeletal myoblasts could respond mechanically to the contraction of surrounding host cardiomyocytes to which they may be bound through the extracellular matrix (25). Alternatively, engrafted myoblasts may release growth factors, particularly insulin-like growth factor-1, with a subsequent paracrine recruitment of cardiac stem cells (26) that would promote a new endogenous pool of contractile cells.

**Study limitations.** A first limitation pertains to the semi-quantitative approach used for assessing segmental function. Although two-dimensional echocardiography has become the technique of choice for direct visualization of endocardial motion and wall thickening (27), this assessment may still be confounded by contractile changes of the adjacent segments, as long as myocardial strain is not directly measured. The improved kinetics of the revascularized segments could therefore have skewed the grading of transplanted areas.

A second limitation is the possible confounding effect of concomitant CABG. Two lines of reasoning, however, temper this concern. First, $^{18}$FDG glucose uptake <50% relative to that of the area of maximal tracer uptake, which defines nonviable scar tissue (14,28), and the lack of contractile response to low-dose dobutamine are both predictable of functional recovery after revascularization, with a negative predictive power in the range of 90% (11,29–32). Thus, at most, 10% of the nonviable segments would have been expected to improve following bypass alone (29–31,33,34), which is in contrast to the observed improvement rate of 63% following transplantation. Second, pathologic studies have shown that nonviable myocardial areas are primarily made of fibrosis (35), with <25% still viable myocytes (36). Because at least 50% of a segment’s myocytes need to be functionally viable to improve following revascularization (36), it is conceivable that the contractile elements that accounted for new-onset systolic thickening of transplanted segments were derived from the grafted myoblasts.

However, the extent to which the improved inotropism of the transplanted segments contributed to the increase in global LVEF remains uncertain. A critical determinant of LVEF recovery is the number of segments with improved wall motion, and this number needs to encompass more than 20% of the LV for global function to improve (37). Combined with revascularization, cell transplantation might contribute to reach this threshold.

**Conclusions.** These data show that autologous skeletal myoblast transplantation is a feasible and straightforward procedure. Randomized trials are now required to further characterize the risk/benefit ratio of this novel approach.

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