Cell Transplantation, Vol. 13, pp. 7–13, 2004
Printed in the USA.

0963-6897/04 $20.00 + .00

www.cognizantcommunication.com

Autotransplantation of Unmanipulated Bone Marrow Into Scarred Myocardium Is Safe and Enhances Cardiac Function in Humans

Manuel Galiñanes,* Mahmoud Loubani,* Joan Davies,† Derek Chin,† John Pasi,‡ and Peter R. Bell§

*Department of Integrative Human Cardiovascular Physiology and Cardiac Surgery, †Department of Cardiology, ‡Division of Haematology, and §Department of Surgery, University of Leicester, Glenfield Hospital, Leicester, LE3 9QP, UK

Stem cell transplants into damaged myocardium may have the potential to improve cardiac function. We investigated the safety of transplanting unmanipulated autologous bone marrow into infarcted myocardium of patients undergoing coronary bypass surgery and assessed its efficacy to improve cardiac function. Fourteen patients with one or more areas of transmural myocardial infarction were studied. Autologous bone marrow was obtained by sternal bone aspirate at the time of surgery, diluted in autologous serum at a ratio of 1:2, and then injected 1 cm apart into the mid-depth of the left ventricular scar. There were no deaths, no perioperative myocardial infarctions, and no significant ventricular arrhythmias. Dobutamine stress echocardiography demonstrated overall improvement in the global and regional left ventricular function 6 weeks and 10 months after surgery. Of 34 infarcted left ventricular segments, 11 were injected with bone marrow alone, 13 were revascularized with a bypass graft alone, and 10 received bone marrow transplantation and a bypass graft in combination. Only the left ventricle segmental wall motion score of the areas injected with bone marrow and receiving a bypass graft in combination improved at low dose and at peak dobutamine stress. These findings suggest that transplantation of unmanipulated autologous bone marrow into scar tissue of the human heart is safe and enhances cardiac function only when used in combination with myocardial revascularization. This benefit can be seen after 6 weeks of the bone marrow transplant and is maintained after 10 months of follow-up.

Key words: Human; Bone marrow; Transplantation; Infarcted myocardium

INTRODUCTION

Damage to the heart muscle following myocardial infarction results in fibrous scarring of the ventricles, remodeling and expansion of the infarct zone, and impairment of pump function. The resultant contractile inefficiency can lead to congestive heart failure, a condition with a prevalence of 3.9% in the general population (13) and an annual mortality of greater than 10% (5). Despite advances in treatment, the management of the patient with end-stage heart failure is difficult. Heart transplantation is restricted by shortage of donors, procedures of cardiomyoplasty and surgical remodeling of the heart are unproven, and the concepts of the total artificial heart or porcine xenograft transplantation remain experimental. An alternative strategy involves the transplantation of cell lines that can differentiate into muscle to restore function or prevent remodeling in the infarcted heart. In this regard, it has been demonstrated in animal models of heart failure that a diverse range of cell types such as skeletal myoblasts (25), bone marrow (15), fetal and neonatal cardiomyocytes (14,22), and even autologous heart cells (9) can improve cardiac function. Recently, it has been reported that the intramuscular injection of autologous myoblasts in a patient with heart failure resulted in significant improvement of cardiac function (11). The use of the patient's own cells for the regeneration of cardiac muscle has the advantages that it is ethically acceptable and immunosuppression is unnecessary. The use of bone marrow cells as opposed to myoblasts and other cell types has the additional benefit of ready availability, without the need for in vitro cell culture preparation.

This nonrandomized study was designed to investigate the safety and potential efficacy of the intramyocardial injection of unmanipulated autologous bone marrow cells into the scarred myocardium of patients undergoing coronary bypass surgery.

Accepted October 20, 2003.
Address correspondence to Professor Manuel Galiñanes, Department of Integrative Human Cardiovascular Physiology and Cardiac Surgery, University of Leicester, Glenfield Hospital, Leicester LE3 9QP, UK. Tel: 0044 116 2563032; Fax: 0044 116 2502449; E-mail: mg50@le.ac.uk

MATERIALS AND METHODS

Patients and Selection Criterion

Ethical approval for this study was obtained from the Leicestershire Health Ethics Committee. Fourteen patients with a Canadian Cardiovascular Society score for angina of ≥2 referred for coronary artery surgery consented to enter the study. Selection criteria included a history of one or more myocardial infarctions and the presence of one or more areas of severe myocardial contractile abnormality (akinesis or dyskinesis) with no contractile improvement during low dobutamine stress. This stress echocardiographic definition of myocardial infarction was confirmed by the presence of fibrosis on the surface of the heart during surgery. All the scarred areas in the left ventricle free wall, but not those in the intraventricular septum, were injected with bone marrow cells. Coronary artery vessels with a stenotic lesion of ≥50% on the angiogram were revascularized with a bypass graft; only those vessels exhibiting a small caliber (e.g., <1 mm in diameter) were considered ungraftable. All 14 patients included in the study received coronary artery bypass grafts as well as autologous bone marrow transplantation. None of the patients had percutaneous angioplasty at any time prior to or after surgery.

Procurement of Bone Marrow and Transplantation

Venous blood (20 ml) was taken from the patient on the morning of surgery using aseptic technique. This was allowed to clot before it was centrifuged at 3000 rpm for 10 min, after which the serum was recovered by aspiration. Standard routine anesthesia was administered to all patients. Bone marrow (5–7 ml) was drawn from the sternum by standard technique prior to sternal split using a standard bone marrow aspiration needle. The aspirated bone marrow was then mixed with the serum at a ratio of 1:2. Heparin was added to the mixture at a final concentration of 50 U/ml. Samples of the mixture were taken for an automated total nucleated cell count as well as enumeration of $CD34^+$ and $CD117^+$ cells by standard flow cytometric analysis. Intraoperative transesophageal echocardiography was performed in all the patients to locate the infarcted area and quantify its thickness. The chest was opened through a median sternotomy and the infarcted area confirmed by direct vision. Core temperature was reduced to 32°C and short periods of intermittent ischemia (12–18 min) interspersed with short periods of reperfusion (3 min) were used to construct each of the coronary anastomoses. At the end of the revascularization procedure the diluted bone marrow was injected into the scarred area of the left ventricle with 250 μl/injection 1 cm apart into the mid-depth of the wall thickness. The inferior wall of the left ventricle was the injected area in all the study patients and in addition the anteroapical area was injected in two of the patients.

Assessment of Perioperative Myocardial Injury

Following surgery all patients were admitted to the cardiac intensive care unit and the heart rhythm was monitored for the first 24 h postoperatively with a 12-lead ECG performed at 6 and 24 h following surgery. Creatine kinase and troponin T were also measured at 6 and 24 h postoperatively using standard assays.

Follow-up

The patients were reviewed at 6 weeks and 10 months postoperatively and questioned regarding angina status and postoperative course.

Dobutamine Stress Echocardiography (DSE)

DSE was performed on all patients prior to surgery and at 6 weeks and 10 months following surgery. Calcium antagonists and β-adrenoceptor antagonists were discontinued 48 h before DSE. Images were acquired at baseline and during infusion of dobutamine at low dose (10 μg/kg/min) and peak stress (maximum dobutamine dose of 40 μg/kg/min).

Digitized images were analyzed by two independent assessors (D.T.C., J.E.D.) who were blinded to the coronary anatomy and the treatment. Using the 16-segment model of the left ventricle, the left ventricular wall motion score index (WMSI) was calculated by scoring each segment (1 = normal, 2 = hypokinesis, 3 = akinesis, and 4 = dyskinesis) and dividing the total score by the number of segments scored. WMSIs were obtained for the whole left ventricle (global WMSI), for the region containing the infarcted areas (regional WMSI), and for the segments of infarction. To increase the specificity for the diagnosis of transmural fibrosis, an infarcted area was defined as an akinetic or dyskinetic segment that does not improve in contractility during low-dose dobutamine stress. The wall thickness for all infarcted segments was measured. Left ventricular ejection fraction, end systolic and end diastolic volumes, and the stroke volume were measured by 2D echocardiography using the biplane method of disks; the cardiac index was assessed by Doppler.

Monitoring of Cardiac Arrhythmia

Surveillance for heart rhythm disturbance included continuous ECG monitoring in the first postoperative 24 h, a 12-lead ECG every day of the postoperative period, and at 6 weeks and 10 months follow-up visit, and a 24-h Holter monitoring study when ambulant at 10 months follow-up.

Statistical Analysis

Data are presented as mean ± SEM. Comparison between WMSI at baseline, 6 weeks, and 10 months was

performed by the repeated measures procedure using the SPSS package (IL). Interobserver agreement in wall motion scores was assessed by calculating the kappa coefficient where $\kappa > 0.4$, $\kappa > 0.6$, and $\kappa > 0.8$ represent a fair, good, and excellent agreement (7); 1923 out of the 2016 (95%) of the LV segments were of sufficient quality for analysis. The interobserver agreement for the independent assessors for wall motion scoring was excellent ($\kappa = 0.83$). Student's *t*-test was used where appropriate. The role of the number of nucleated and $CD34^+/CD117^+$ cells injected in contractility changes was analyzed by multiple regression analysis. The rationale for the measurement of $CD34^+/CD117^+$ cells was based on the report by Orlic et al. (15), where they selected hemopoietic precursors to treat mice with myocardial infarction (see below). A value of $p < 0.05$ was taken as significant.

RESULTS

The clinical characteristics of the patients participating in the study are presented in Table 1. All patients had significant angina and dyspnea. All the patients were diagnosed with triple vessel coronary artery disease and had impaired global left ventricular function. The mean period interval between the occurrence of myocardial infarction and surgery for the study population was 23 ± 17 months. A mean of 2.2 ± 0.2 coronary artery anastomoses was constructed. Table 1 shows the operative data.

Table 1. Preoperative and Operative Data and Postoperative Complications

Preoperative data	
Age	62.1 ± 2.3
Male/female ratio	12:2
Angina CCS class (range 2–4)	3 ± 0.2
Dyspnea NYHA class (range 1–4)	2.1 ± 0.2
Congestive heart failure	4/14
Diabetes mellitus	5/14
Hypercholesterolemia	10/14
Hypertension	8/14
Atrial fibrillation	1/14
Ejection fraction	
<30%	4
30–50%	9
>50%	1
Parsonnet score	7.4 ± 1.4
Euroscore	4 ± 0.6
Operative data	
Aortic cross clamp time (min)	33.3 ± 2.9
Cardiopulmonary bypass time (min)	64.2 ± 5.5
Postoperative complications	
Patients requiring inotropes	2/14
Atrial fibrillation	2/14
Pneumothorax	1/14
Chest infection	1/14

CCS: Canadian Cardiovascular Society, NYHA: New York Heart Association.

A mean of 26 ± 2 injections (range 16–44) of unmanipulated bone marrow was applied to the scarred left ventricular wall, causing an increase in wall thickness at the site of injection from 0.74 ± 0.04 to 0.84 ± 0.04 cm ($p < 0.05$) measured by transesophageal echocardiography. There were no immediate perioperative complications. The postoperative complications listed in Table 1 were not of a type that could attribute causation to the bone marrow injections. The mean stay of patients in the hospital was 7.0 ± 0.6 days. There were no new electrocardiographic changes or significant elevation in creatine kinase (475 ± 77 and 472 ± 31 IU/L at 6 and 24 h after the termination of surgery) or troponin T (1.5 ± 0.2 and 0.8 ± 0.2 ng/ml at 6 and 24 h after the termination of surgery) to indicate perioperative myocardial infarction. There were no deaths, new myocardial infarctions, or other cardiac events during the first 10 months of follow-up and all patients improved clinically; the angina Canadian Cardiovascular Society class and dyspnea New York Heart Association class mean values were reduced from 3.0 ± 0.2 and 2.1 ± 0.2 before surgery to 1.1 ± 0.1 and 1.2 ± 0.1 at 6 weeks and to 0.3 ± 0.1 and 1.2 ± 0.1 at 10 months follow-up ($p < 0.05$ in both instances). A 24-h Holter performed at 10 months' follow-up did not show significant ventricular arrhythmias. These results demonstrate that transplantation of unmanipulated autologous bone marrow by intramyocardial injections during heart surgery is safe for the duration of the study and that it is not associated with perioperative complications or detrimental medium-term effects. They provide reassurance in light of in vitro evidence that stem cell-derived cardiomyocytes have the potential to generate arrhythmias (30). Liver and renal function tests were not performed during the follow-up period; however, none of the patients presented with hepatic or renal complications.

Echocardiography showed that prior to surgery patients had significant left ventricular dysfunction with a mean global WMSI of 1.97 ± 0.11 at rest. With dobutamine stress, there was a biphasic change in WMSI (1.68 ± 0.1 at low dose and 1.91 ± 0.09 at peak dose). This reflects the overall presence of hibernating myocardium that would respond to revascularization alone. In keeping with this, Figure 1a shows improvement in global WMSI at 6 weeks (1.94 ± 0.01 at rest vs. 1.64 ± 0.11 at low dose and 1.61 ± 0.11 at peak dose; $p < 0.05$) and at 10 months (1.76 ± 0.10 at rest vs. 1.64 ± 0.11 at low dose and 1.66 ± 0.11 at peak dose; $p < 0.05$) after surgery. The mean value for left ventricular ejection fraction at rest before surgery was 47 ± 4% (this value was obtained by biplane method of disks and contrasts

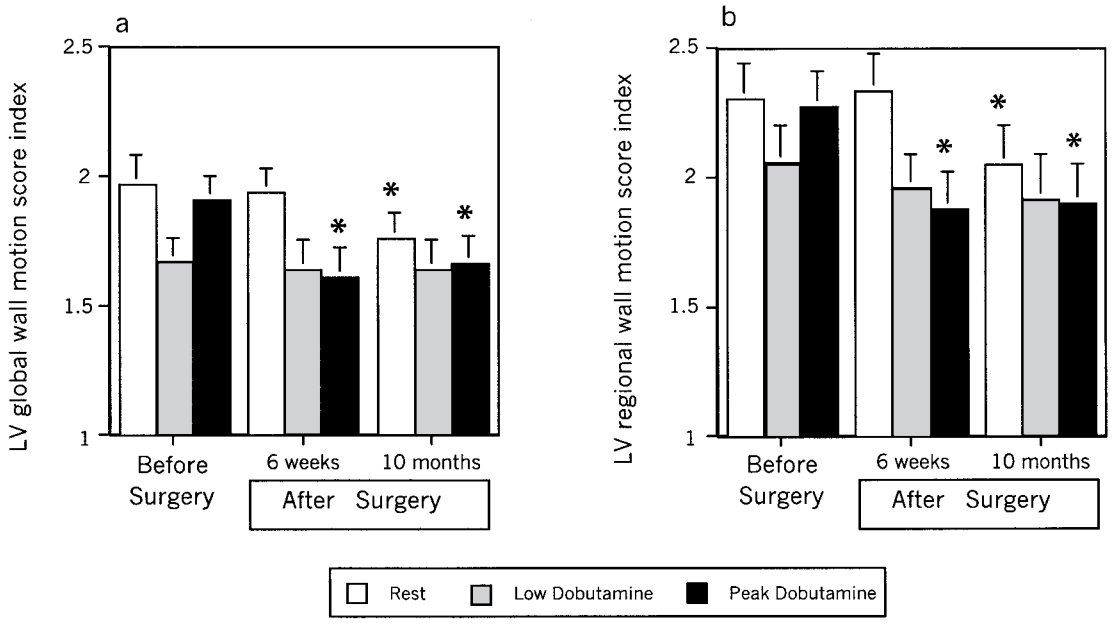

Figure 1. Left ventricle (LV) global (a) and regional (b) wall motion score index before surgery and 6 weeks and 10 months after surgery at rest and at low and peak dobutamine stress in the entire study population (14 patients). *$p < 0.05$ versus the corresponding mean values before surgery.

with the left ventricular ejection fraction of 38 ± 2% measured by single plane left ventriculography). There was no significant difference in left ventricular ejection fraction at rest before and after surgery (44 ± 4% at 6 weeks and 48 ± 4% at 10 months); however, there was a significant improvement of the left ventricular ejection response to dobutamine stress following surgery (47 ± 2% before surgery vs. 58 ± 4% at 6 weeks and 55 ± 4% at 10 months at peak dose; $p < 0.05$). The end systolic and end diastolic volumes and the stroke volume index at rest echocardiography were not significantly altered at any time period (57.0 ± 9.0 vs. 54.3 ± 8.7 and 55.9 ± 8.7 ml; 95.9 ± 9.5 vs. 87.0 ± 11.1 and 98.8 ± 10.7 ml; 35.9 ± 1.5 vs. 32.0 ± 1.7 and 33.5 ± 1.2 ml/m^2, respectively). On the other hand, the improvement in the cardiac index seen with low and peak dobutamine stress prior to surgery (2.7 ± 0.2 vs. 4.0 ± 0.3 and 4.3 ± 0.3l/min/m^2; $p < 0.05$) was also observed at 6 weeks (2.6 ± 0.2 vs. 4.0 ± 0.3 and 4.8 ± 0.2l/min/m^2; $p < 0.05$) and at 10 months following surgery (2.6 ± 0.2 vs. 3.6 ± 0.3 and 4.5 ± 0. 3l/min/m^2; $p < 0.05$).

To assess the impact of the bone marrow injections on regional function, we analyzed the wall motion score index in the ventricular walls containing injected infarct segments (regional WMSI). Figure 1b shows that the resting regional WMSI changed from 2.32 ± 0.12 before surgery to 2.35 ± 0.13 at 6 weeks and 2.07 ± 0.15 at 10 months, a significant improvement ($p < 0.05$). However, the regional WMSIs of between 2 and 3 indicate that the areas under study may have contained both fibrotic tissue (i.e., akinetic) and viable myocardium (i.e., hypokinetic). The coexistence of fibrotic and viable myocardium in the regional analyses of contractile function is further supported by the biphasic response of the regional WMSI prior to surgery, improving from 2.32 to 2.06 at low-dose stress and deteriorating to 2.28 at peak stress. Interestingly, the deterioration of regional WMSI seen before surgery was reversed at 6 weeks and 10 months after surgery.

The 34 infarcted segments in the 14 study patients were divided into three groups according to the treatment applied: (i) bone marrow injection alone ($n = 11$ segments), (ii) bypass graft alone ($n = 13$ segments), and (iii) bone marrow injection and bypass graft in combination ($n = 10$ segments). Figure 2 shows that, as expected, the infarcted segments are predominantly akinetic, as the mean values for the segmental WMSI in the three groups were 3.0 ± 0.0, 3.0 ± 0.0, and 3.1 ± 0.1, respectively, and there was no contractile improvement with dobutamine stress. Importantly, whereas bone marrow transplantation and bypass graft alone did not ameliorate segmental WMSI, the combination of the two treatment modalities resulted in statistically significant improvement of the scores at low and peak dobutamine stress both at 6 weeks and 10 months after surgery, which may indicate an increase in contractile tissue in these segments. However, the improvement in contractility was not matched by a significant increase in wall thickness

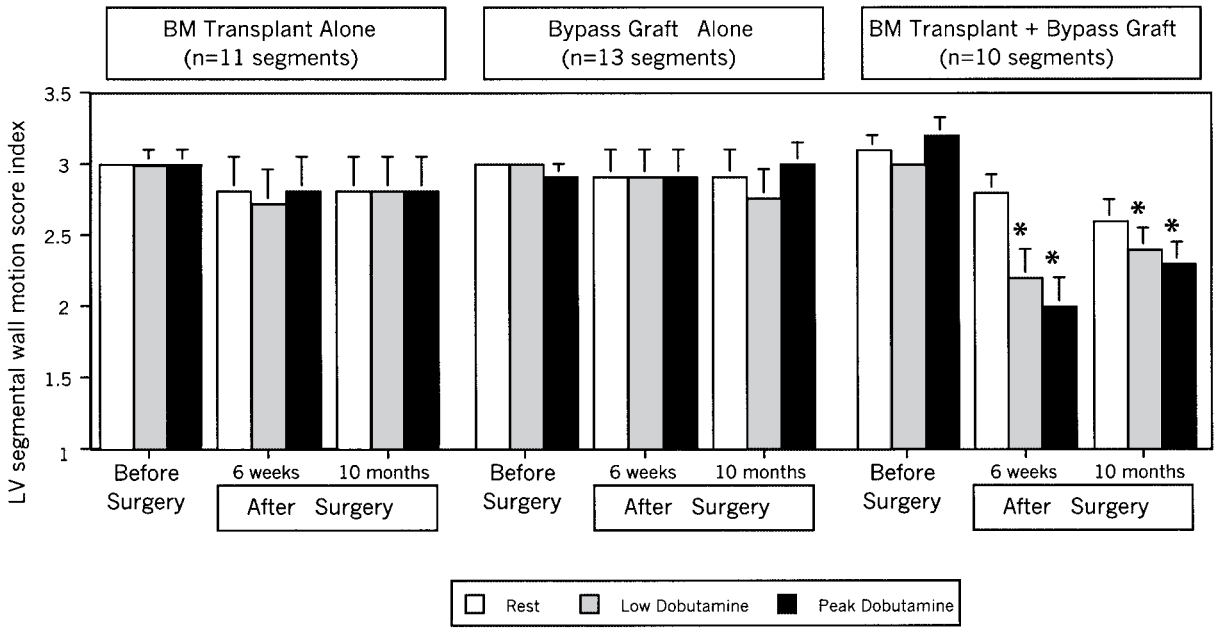

Figure 2. Left ventricle (LV) segmental wall motion score index before surgery and 6 weeks and 10 months after surgery at rest and at low and peak dobutamine stress of infarcted segments that received injection of bone marrow alone ($n = 11$ segments), bypass graft alone ($n = 13$ segments), or bone marrow and bypass graft in combination ($n = 10$ segments). $*p < 0.05$ versus the corresponding mean values before surgery.

of all the infarcted segments (0.88 ± 0.04 and 0.86 ± 0.04 cm at 6 weeks and at 10 months after surgery vs. 0.86 ± 0.04 cm before surgery; $p = \text{NS}$) and of the segments injected with bone marrow (0.92 ± 0.06 and 0.88 ± 0.05 cm at 6 weeks and at 10 months after surgery vs. 0.86 ± 0.07 cm before surgery; $p = \text{NS}$) as measured by transthoracic echocardiography. Similarly, there was no correlation between the length of the period interval of myocardial infarctions and the segmental WMSI.

The number of mononuclear cells and dual staining $CD34^+/CD117^+$ ($c\text{-}kit^{pos}$) injected per each treated segment was $31.5 \pm 3.5 \times 10^6$ and $0.61 \pm 0.1 \times 10^6$, respectively, and they were not correlated with the improvement in contractility. Previous studies have demonstrated that the putative pluripotential stem cell is $Lin^- c\text{-}kit^{pos}$ in murine models of cardiac regeneration (15). These cells exist at a low level within the bone marrow somewhere between 1 in 10,000 to 100,000 cells and it is reasonable to assume that only a relatively small number of true pluripotent stem cells has been transplanted at each injection site in our studies.

DISCUSSSION

The present study has demonstrated that transplantation of unmanipulated autologous bone marrow into scar tissue of the human heart is safe and has shown for the first time that it enhances cardiac function only when used in combination with myocardial revascularization. The differentiation of bone marrow cells into myocytes, endothelial cells, and other tissue components necessary to support the viability and function of the contractile units is probably the mechanism underlying the improvement of contractile function by bone marrow transplantation in our study. Indeed, it was shown as early as 1968 that the bone marrow possessed pluripotential stem cells, which under appropriate experimental conditions can differentiate into a broad spectrum of tissues (2). More recent experiments in animal preparations have shown that bone marrow-derived progenitor cells are of enormous plasticity so that they can differentiate into myocardium (10,15,27), brain tissue (12), liver (17), skeletal muscle (1), vessels (21), and other tissues. Human mesenchymal stem cells were first isolated and shown to have multiple differentiation potential by Haynesworth et al. (4). Thereafter, the multilineage potential of human mesenchymal cells was demonstrated by in vitro methods (18) and that these cells can differentiate into cardiomyocytes when injected in the left ventricle of the adult murine heart (26). Recently it has been suggested that bone marrow cells induce angiogenesis when injected into the infarct border zone in patients undergoing coronary bypass grafting (28) and into the ischemic myocardium (23). All the evidence suggests that bone marrow is a potential inexhaustible source of pluripotential cells that can differentiate into a variety of cell types dependent on local environmental cues. For ethical reasons

and technical constraints, the possible differentiation of bone marrow cells into vascular and contractile tissue was not investigated in the present study; however, our finding that the improvement in contractility of the areas injected with bone marrow is only apparent when combined with bypass grafting suggests that the improvement on muscle contraction requires normalization of the blood supply. The results may imply that the transplantation of bone marrow cells into nonrevascularized myocardium lose the capacity to regenerate contractile structures or that they are not resilient to survive in a hypoxic environment.

The $CD34^+/CD117^+$ counts are a simplistic analysis of possible stem cell number in the samples and will represent a significant overestimation of the true number of pluripotent stem cells. Furthermore, the bone marrow taken and injected would contain a mixture of hemopoeitic stem cell (HSC), mesenchymal stem cells (MSC), and other undefined stem cells. Both HSC and MSC have now been shown to have significant capacity to differentiate into a variety of cell types (1,6,8,17, 19). However, it has been recently suggested that HSC transdifferentiation into nonhemopoietic cell lines is not common in the steady state (29), although the response in the setting of selective tissue, whether this is normal or injured, is not known. While the debate over the specific capacity of HSCs to transdifferentiate remains unresolved, published data clearly suggest that marrow-derived cells, whether distinct tissue specific or truly pluripotent, can differentiate into a variety of cell types, a view supported by the clinical observation of this study. It is of interest that in the present study the total number of mononuclear cells or $CD34^+/CD117^+$ cells injected was not correlated with the improvement in contractility. This observation probably represents the fact that such surrogate markers do not accurately reflect the numbers of stem cells injected as they exist at a very low frequency in the marrow. Further experiments will be required to determine the full developmental capacity and origin of such marrow-derived stem cells.

Clearly, the mechanism, origin (HSC, MSC, or other undefined), and the identification of the phenotype of the marrow-derived cells responsible for the reconstitution of heart tissue need to be investigated. However, the results of our study suggest that, while these questions are addressed, there is no obvious impediment to proceed with further studies in humans to refine the treatment and to identify the group of patients that may benefit from this therapy. Thus, for example, whether the number of bone marrow cells required for improving contractility is an important factor and whether one or more transplants are needed to produce enough muscle mass to attain meaningful clinical results must be elucidated before a wider clinical application of this therapy is considered. Moreover, direct injection of the bone marrow into the muscle may be an inefficient and impractical route of cell delivery and it is not known whether the intravascular and intracoronary administrations are a better option. Recently it has been reported that the transendocardial transplantation of bone marrow cells is safe in subjects with advanced coronary artery disease (3) and with chronic ischemic heart failure (16) and also that the selective intracoronary transplantation of autologous mononuclear bone marrow cells is safe and effective in the context of acute (24) myocardial infarction.

Despite the obvious importance of our results, it should be emphasized that this was a nonrandomized study involving a small number of patients, and that the findings should be confirmed in a larger randomized study. It is also worth noting that although the intramyocardial injection of autologous bone marrow is safe for the first 10 months following its application, longer follow-up periods would be required to ascertain that the treatment does not induce long-term adverse events.

In conclusion, we have shown that transplantation of unmanipulated autologous bone marrow may provide a novel and promising means of reversing postinfarction left ventricular dysfunction and may have important clinical implications for improving the long-term prognosis of these patients and possibly the prevention of the occurrence of heart failure. The use of bone marrow avoids the complexity of cell culture techniques and the concerns that repeated in vitro passaging of primary cells like myoblasts may alter their ability to fuse and differentiate (20). Furthermore, in comparison to transplanted allogenic and xenogenic cells, there is no risk of rejection or requirement for immunosuppressant drugs.

ACKNOWLEDGMENTS: This work was supported by the personal contribution of the authors. We are grateful to Mrs. Helen Spence for her help on the preparation of the manuscript.

REFERENCES

1. Ferrari, G.; Cusella-De Angelis, G.; Coletta, M.; et al. Muscle regeneration by bone marrow-derived myogenic progenitors. Science 279:1528–1530; 1998.
2. Friedenstein, A. J.; Petrakova, K. V.; Kurolesova, A. I.; Frolova, G. P. Heterotopic of bone marrow: Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation 6:230–247; 1968.
3. Fuchs, S.; Satler, L. F.; Kornowski, R.; et al. Catheter-based autologous bone marrow myocardial injection in no-option patients with advanced coronary artery disease. J. Am. Coll. Cardiol. 41:1721–1724; 2003.
4. Haynesworth, S. E.; Goshima, J.; Goldberg, V. M.; Caplan, A. I. Characterization of cells with osteogenic potential from human marrow. Bone 13:81–88; 1992.
5. Ho, K. K.; Anderson, K. M.; Kannel, W. B.; Grossman, W.; Levy, D. Survival after the onset of congestive heart failure in Framingham Heart Study subjects. Circulation 88:107–115; 1993.

6. Kopen, G. C.; Prockop, D. J.; Phinney, D. G. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc. Natl. Acad. Sci. USA 96:10711–10716; 2000.
7. Kramer, M. S.; Feinstein, A. R. Clinical biostatistics: The biostatistics of concordance. Clin. Pharmacol. Ther. 29: 111–123; 1981.
8. Krause, D. S.; Theise, N. D.; Collector, M. I.; et al. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 105:369–377; 2001.
9. Li, R. K.; Weisel, R. D.; Mickle, D. A.; et al. Autologous porcine heart cell transplantation improved heart function after a myocardial infarction. J. Thorac. Cardiovasc. Surg. 119:62–68; 2000.
10. Makino, S.; Fukuda, K.; Miyoshi, S.; et al. Cardiomyocytes can be generated from marrow stromal cells in vitro. J. Clin. Invest. 103:697–705; 1999.
11. Menasche, P.; Hagege, A. A.; Scorsin, M.; et al. Myoblast transplantation for heart failure. Lancet 357:279–280; 2001.
12. Mezey, E.; Chandross, K. J.; Harta, G.; Maki, R. A.; McKorcher, S. R. Turning blood into brain: Cells bearing neuronal antigens generated in vivo from bone marrow. Science 290:1779–1782; 2000.
13. Mosterd, A.; Hoes, A. W.; de Bruyne, M. C.; et al. Prevalence of heart failure and left ventricular dysfunction in the general population. Eur. Heart J. 20:447–455; 1999.
14. Muller-Ehmsen, J.; Peterson, K. L.; Kedes, L.; et al. Rebuilding a damaged heart: Long-term survival of transplanted neonatal rat cardiomyocytes after myocardial infarction and effect on cardiac function. Circulation 105: 1720–1726; 2002.
15. Orlic, D.; Kajstura, J.; Chimenti, S.; et al. Bone marrow cells regenerate infarcted myocardium. Nature 410:701–705; 2001.
16. Perin, E. C.; Dohmann, H. F. R.; Borojevic, R.; et al. Transendocardial autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. Circulation 107:2294–2302; 2003.
17. Petersen, B. E.; Bowen, W. C.; Patrene, K. D.; et al. Bone marrow as a potential source of hepatic oval cells. Science 284:1168–1170; 1999.
18. Pittenger, M. F.; Mackay, A. M.; Beck, S. C.; et al. Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147; 1999.
19. Reyes, M.; Dudek, A.; Jahagindar, B.; Koodie, L.; Marker, P. H.; Verfaillie, C. Origin of endothelial progenitors in human postnatal bone marrow. J. Clin. Invest. 109: 337–346; 2002.
20. Rosenblatt, J. D.; Lunt, A. I.; Parry, D. J.; Partridge, T. A. Culturing satellite cells from living single muscle fiber explants. In Vitro Cell Dev. Biol. Anim. 31:773–779; 1995.
21. Shintani, S.; Murohara, T.; Ikeda, H.; et al. Augmentation of postnatal neovascularization with autologous bone marrow transplantation. Circulation 103:897–903; 2001.
22. Soonpaa, M. H.; Koh, G. Y.; Klug, M. G.; Field, L. J. Formation of nascent intercalated disks between grafted fetal cardiomyocytes and host myocardium. Science 264: 98–101; 1994.
23. Stamm, C.; Westphal, B.; Kleine, H. D.; et al. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. Lancet 361:45–46; 2003.
24. Strauer, B. E.; Brehm, M.; Zeus, T.; et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 106:1913–1918; 2002.
25. Taylor, D. A.; Atkins, B. Z.; Hungspreugs, P.; et al. Regenerating functional myocardium: Improved performance after skeletal myoblast transplantation. Nat. Med. 4:929–933; 1998.
26. Toma, C.; Pittenger, M. F.; Cahill, K. S.; Byrne, B. J.; Kessler, P. D. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. Circulation 105:93–98; 2002.
27. Tomita, S.; Li, R. K.; Weisel, R. D.; et al. Autologous transplantation of bone marrow cells improves damaged heart function. Circulation 100(Suppl.):II247–II256; 1999.
28. Tse, H. F.; Kwong, Y. L.; Chan, J. K.; Lo, G.; Ho, C. L.; Lau, C. P. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. Lancet 361:47–49; 2003.
29. Wagers, A. J.; Sherwood, R. I.; Christensen, J. L.; Weissman, I. L. Little evidence for developmental plasticity of adult hematopoietic stem cells. Science 297:2256–2259; 2002.
30. Zhang, Y. M.; Hartzell, C.; Narlow, M.; Dudley, S. C. Stem cell-derived cardiomyocytes demonstrate arrhythmic potential. Circulation 106:1294–1299; 2002.