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EDITORIAL COMMENT

Skeletal Muscle Meets Cardiac Muscle

Friends or Foes?*

Elina Minami, MD,†‡ Hans Reinecke, PHD,† Charles E. Murry, MD, PHD†
Seattle, Washington

Since the early 1990s, cellular cardiomyoplasty (or simply cell transplantation) has been explored as a potential new therapy to alleviate the consequences of myocardial infarction (MI) (1). Many different cell types have been tried (2) and, quite naturally, cardiomyocytes were chosen early on, reasoning that they would be the ideal replacement for lost cardiomyocytes (3,4). Since these groundbreaking studies, several investigators have shown that cardiomyocytes can form viable grafts in and integrate with the host myocardium (5–8). However, there are several significant drawbacks to this cell type. The first problem stems from availability, that is, for today's infarct patient and likely for the next several years, there is no feasible source of cardiomyocytes for clinical applications. Embryonic stem cells show the most promise for large scale cardiomyocyte production, but much basic research remains to be done with these cells before they could be used safely or effectively.

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The ethics of embryonic stem cell use are also being debated in multiple arenas, indicating it will be some years before these cells can be used for treating patients. Adult stem cells (such as hematopoietic stem cells) circumvent the ethical constraints of embryonic stem cells, but their more limited plasticity makes generation of cardiomyocytes a very inefficient process at present. The second problem with cardiomyocytes is that they are not a very hardy cell type. Cardiomyocytes are quite ischemia-sensitive and die in large numbers (up to 99%) after being grafted into a hostile environment such as a healing infarct or an old scar (7,9). Thus, while one can make new myocardium by cardiomyocyte transplantation, it is very difficult to create it in physiologically relevant amounts.

Skeletal muscle transplantation for cardiac repair. A way out of this dilemma was shown with the use of stem cells from skeletal muscle, so-called myoblasts or satellite cells. Skeletal myoblasts are the mononucleated precursors to skeletal myofibers, and, after injury, these normally quiescent cells proliferate and then fuse to regenerate new multinucleated myofibers. The great advantage of using myoblasts lies in the fact that these cells can be isolated from the patient's own skeletal muscle, expanded in vitro, and transplanted back into the patient's heart, thus circumventing any histocompatibility and/or ethical concerns involved with the use of some other candidate cell types. In 1996, our group found that skeletal myoblasts not only survived after grafting into an injured heart, but also proliferated for several days and then differentiated into mature myofibers (10). Because of their increased survival and proliferative capacity, much bigger grafts of skeletal muscle can be generated by cell implantation. Phenotypically, the myofibers were similar to fast-twitch skeletal muscle, but as wound healing progressed, some fibers developed characteristics of slow twitch muscle, for example, expression of slow beta-myosin heavy chain. Fiber conversion was interpreted as potentially beneficial to the heart because slow fibers are much more fatigue-resistant and, thus, more suitable to sustain a cardiac-type workload. Interestingly, there was also some evidence for the establishment of a new satellite cell, that is, stem-cell population. Skeletal muscle grafts in the heart will contract when exogenously stimulated (10). However, they do not form electromechanical junctions with cardiomyocytes due to the absence of intercalated disk proteins (11). Thus, it is not clear whether skeletal muscle grafts actually beat in vivo. This question remains one of the major goals for basic scientists to answer.

Altogether, over 45 papers investigating skeletal myoblast grafting for cardiac repair in various small and large animal models have been published. Although space limitations preclude a detailed review of their results, a few deserve special attention. Considerable excitement resulted from the work of Taylor et al. (12) who, for the first time, demonstrated that autologous skeletal myoblast grafting into cryoinjured rabbit hearts improved regional myocardial performance in vivo. Then, in 2001, Jain et al. (13) found evidence that syngeneic myoblast implantation after MI improves both in vivo and ex vivo indexes of global ventricular dysfunction after MI. Although infarct sizes were comparable in the two groups, hearts with skeletal myoblast grafts had significantly less ventricular remodeling. Thus, prevention of remodeling is one potential mechanism by which myoblast grafting can improve function of the infarcted heart.

Skeletal muscle cell grafting for cardiac repair appears to have come of age and, finally, 10 years after the initial report from Marelli et al. (1), to the clinic. In this issue of the *Journal*, Menasché et al. (14) describe the outcome of the first clinical trial using skeletal myoblasts for infarct repair. For those readers who have not followed this field closely, it should be mentioned that the Dr. Menasché's group is well-established in the cellular cardiomyoplasty field, having published numerous significant studies in preclinical models

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From the Department of Pathology and Division of Cardiology, University of Washington, Seattle, Washington. Dr. Murry has sponsored research with Geron, Menlo Park, California.

of infarct repair. For example, they were the first to directly compare the beneficial effects of cardiomyocyte versus skeletal myoblast grafting in a rat infarct model, finding that skeletal myoblasts were just as effective as fetal cardiomyocytes for improving postinfarction left ventricular (LV) function (15). Subsequently, they found a significant linear relationship between the improvement in ejection fraction and the number of injected skeletal myoblasts after transplantation. They concluded that autologous myoblast transplantation is functionally effective over a wide range of postinfarct ejection fractions, including the sickest hearts, provided that they are injected with a sufficiently high number of cells (16). Most importantly, in 2001 they reported the first human case study where they implanted autologous skeletal myoblasts into the postinfarction scar during coronary artery bypass grafting (CABG) of remote myocardial areas. Five months after implantation, there was evidence of contraction and viability in the grafted scar by echocardiography and positron emission tomography. Although encouraging, the authors were cautious enough to emphasize the need for validation by additional studies (17). With the history of this laboratory in mind, the current phase I trial seems a logical extension of this systematic approach.

Overview of the phase I trial. Based on the promise of the preclinical studies and the single case report, Menasché et al. (14) provide a much-awaited clinical follow-up. They describe the first phase I clinical trial looking at the feasibility and safety of autologous skeletal myoblast transplantation in patients with severe ischemic cardiomyopathy. Ten heart failure patients were selected on the basis of: 1) having an old MI, and 2) needing CABG in areas remote from the previous infarct. These patients underwent skeletal muscle biopsy from the thigh, and myoblasts were isolated from the biopsies for direct injection into infarcted myocardium during bypass surgery. The infarcted regions were determined as areas that lacked response to low-dose dobutamine and had ^{18}fluoro-2-deoxyglucose uptake of $<$50% that of control myocardium by positron emission tomography imaging. All patients underwent pre- and postprocedural echocardiographic analysis for LV function. Because this is a phase I trial, the primary end points were feasibility and safety of the procedure, and the secondary end point was efficacy, which was measured primarily as echocardiographic improvement of the injected sites.

Is skeletal myoblast grafting feasible and safe? Human myocardium contains ~20 million cardiomyocytes per g. If an LV weighs ~250 g, and a failure-inducing infarct kills off 30% to 40% of the ventricle, this translates to a deficiency of 750 million to 1 billion cardiomyocytes. Thus, the first goal was to determine if a physiologically significant number of skeletal myoblasts could be generated from biopsies in a timely fashion. The authors report that autologous skeletal myoblast grafting is, indeed, quite feasible. They set goals of achieving at least 500 million cells, with a purity of at least 60% myoblasts, within two to three weeks of biopsy. They easily reached (and generally overshot) these goals, and cell viability was excellent. Hearts received an average of 37 injections under cardioplegic arrest, delivering an average of 871 million cells in 5.7 ml to a mean area of 29 cm^2. Menasché et al. (14) point out that the optimal cell number for injection is still unknown, but based on their animal data (16), they have demonstrated that cell number correlates well with cardiac functional improvement. It is clear that dose-escalation studies will be required to define the optimal cell dose for human myoblast grafting.

For any new clinical intervention, safety is obviously of paramount importance. One of the 10 patients died acutely from complications unrelated to myoblast injection. Of the remaining nine patients, four developed monomorphic ventricular tachycardia, one of which included a syncopal event. These four patients eventually required placement of automatic implantable cardioverter-defibrillator. As of this writing, one of the four patients has received two appropriate shocks, while the other three patients have not required intervention. Were these arrhythmias related to myoblast implantation? The small size of this trial and the absence of a control group preclude a definitive answer. The authors, however, are appropriately cautious about this complication, stating that the possibility of a direct connection must be explored further. Their current protocol involves prophylactic administration of amiodarone for three months postoperatively. The possible complication of arrhythmias raises additional questions. Will all patients require a defibrillator and antiarrhythmic therapy? What would be the added cost? Additional studies and longer follow-up need to be performed to determine whether there is a significantly higher risk of developing ventricular arrhythmias after this intervention.

Is skeletal myoblast grafting effective? Menasché et al. (14) stress that this was a feasibility and safety study, neither designed nor powered to test efficacy. Furthermore, they emphasize that the concomitant CABG is a variable that could confound interpretation of functional data. With these limitations in mind, they attempted a careful assessment of cardiac performance. Left ventricular ejection fraction significantly improved from 23.8% to 32.1% and New York Heart Association (NYHA) functional class from 2.7 to 1.6. These findings are consistent with preclinical animal data on cell transplantation, but also could be explained by revascularization therapy. To examine effects of grafting more directly, they examined segmental wall motion by echocardiography. By correlating the intraoperative diagrams of cell implantation with echocardiograms, they estimated that a total of 22 myocardial segments received myoblast implants. Of these, 14 segments (from six of eight patients) demonstrated significant improvement in systolic motion when preoperative and postoperative studies were compared in a blinded fashion. Menasché et al. (14) point out the limitations of using echocardiography as a measure for cardiac functional improvement because there is no way to discriminate whether improvement is a result of passive

motion from nearby segments enhanced by surgical revascularization alone or, alternatively, through increased collateral flow to the infarcted region. However, they injected the cells in scarred areas and point out that the observed improvement is out of proportion to what is expected with surgical revascularization alone. Thus, they provide tantalizing evidence that myoblast grafts are improving ventricular function. The mechanism for such a functional improvement with cell injection still remains a paradox, especially because differentiated skeletal muscle fibers lack gap junctions in vivo to allow for synchronized, mechanical coupling with cardiomyocytes (11). It would be helpful to know to what extent the ^{18}FDG uptake improved with cell injection and whether all of the successfully injected regions also had functional improvement. In addition, it would be interesting to determine whether functional and NYHA class improvement are associated with any change in maximal exercise capacity and maximal oxygen consumption during exercise.

Summary and future directions. This trial is a significant step forward in the area of cell transplantation. We commend the authors for their careful approach to developing this therapy and for the thoughtful, self-critical analysis that they have provided. As the first application of cell-based, regenerative medicine to human heart disease, this study sets a standard for how cell-based therapies can be moved safely to the clinic. This trial was based on many dedicated years of research that first showed promise in rodents and then was scaled up to larger animals. The authors did not rush to clinical trials, but rather worked to establish important proofs of principle in relevant preclinical models. We hope that other investigators working in cell-based therapeutics will take a similarly reasoned approach. This phase I trial has opened our eyes to the possibility of arrhythmic complications and raises the possibility of functional improvement. The stage is now set for placebo-controlled, multicentered trials that will more definitively assess the safety and efficacy of myoblast transplantation for infarct repair.

Reprint requests and correspondence: Dr. Charles E. Murry, University of Washington, Department of Pathology, Box 357470, Room D-514 HSB, Seattle, Washington 98195-7335. E-mail: murry@u.washington.edu.

REFERENCES

1. Marelli D, Desrosiers C, el-Alfy M, Kao RL, Chiu RC. Cell transplantation for myocardial repair: an experimental approach. Cell Transplant 1992;1:383–90.
2. Muller-Ehmsen J, Kedes LH, Schwinger RH, Kloner RA. Cellular cardiomyoplasty—a novel approach to treat heart disease. Congest Heart Fail 2002;8:220–7.
3. Koh GY, Soonpaa MH, Klug MG, Field LJ. Long-term survival of AT-1 cardiomyocyte grafts in syngeneic myocardium. Am J Physiol 1993;264:H1727–33.
4. Soonpaa MH, Koh GY, Klug MG, Field LJ. Formation of nascent intercalated disks between grafted fetal cardiomyocytes and host myocardium. Science 1994;264:98–101.
5. Reinecke H, Zhang M, Bartosek T, Murry CE. Survival, integration, and differentiation of cardiomyocyte grafts: a study in normal and injured rat hearts. Circulation 1999;100:193–202.
6. Etzion S, Battler A, Barbash IM, et al. Influence of embryonic cardiomyocyte transplantation on the progression of heart failure in a rat model of extensive myocardial infarction. J Mol Cell Cardiol 2001;33:1321–30.
7. Muller-Ehmsen J, Whittaker P, Kloner RA, et al. Survival and development of neonatal rat cardiomyocytes transplanted into adult myocardium. J Mol Cell Cardiol 2002;34:107–16.
8. Muller-Ehmsen J, Peterson KL, 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 2002;105:1720–6.
9. Zhang M, Methot D, Poppa V, Fujio Y, Walsh K, Murry CE. Cardiomyocyte grafting for cardiac repair: graft cell death and anti-death strategies. J Mol Cell Cardiol 2001;33:907–21.
10. Murry CE, Wiseman RW, Schwartz SM, Hauschka SD. Skeletal myoblast transplantation for repair of myocardial necrosis. J Clin Invest 1996;98:2512–23.
11. Reinecke H, MacDonald GH, Hauschka SD, Murry CE. Electromechanical coupling between skeletal and cardiac muscle: implications for infarct repair. J Cell Biol 2000;149:731–40.
12. Taylor DA, Atkins BZ, Hungspreugs P, et al. Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. Nat Med 1998;4:929–33.
13. Jain M, DerSimonian H, Brenner DA, et al. Cell therapy attenuates deleterious ventricular remodeling and improves cardiac performance after myocardial infarction. Circulation 2001;103:1920–7.
14. Menasché P, Hagège AA, Vilquin J-T, et al. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. J Am Coll Cardiol 2003;41:1078–83.
15. Scorsin M, Hagege A, Vilquin JT, et al. Comparison of the effects of fetal cardiomyocyte and skeletal myoblast transplantation on postinfarction left ventricular function. J Thorac Cardiovasc Surg 2000;119:1169–75.
16. Pouzet B, Vilquin JT, Hagege AA, et al. Factors affecting functional outcome after autologous skeletal myoblast transplantation. Ann Thorac Surg 2001;71:844–51.
17. Menasché P, Hagége AA, Scorsin M, et al. Myoblast transplantation for heart failure. Lancet 2001;357:279–80.