Journal of the American College of Cardiology
© 2008 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 52, No. 23, 2008
ISSN 0735-1097/08/$34.00
doi:10.1016/j.jacc.2008.07.066

Skeletal Myoblasts for Cardiac Repair: Act II?*

Philippe Menasché, MD, PHD

Paris, France

Ten years ago, when the increased prevalence of heart failure along with the recognition of the limitations of existing therapies have forced the consideration of new therapeutic options, implantation of contractile cells into scarred myocardium has emerged as an attractive strategy. This approach was actually backed by the long-standing successful outcomes of bone marrow and skin transplantation that first rationalized the use of cells as therapeutic agents. Although it was recognized that the most logical approach would have been to use cardiac cells, this turned out not to be possible: fetal cardiomyocytes raised complicated ethical, logistical, and technical issues (1); there was (and still there is) no means for effectively mobilizing a hypothetical pool of resident stem cells (2); and research on embryonic stem cell-derived cardiac progenitors was still in infancy. Investigators then looked at cells that might act as

See page 1869

cardiomyocyte surrogates, and, in this setting, autologous skeletal myoblasts were found clinically attractive because of the easiness of procurement, their in vitro scalability, and the lack of serious concerns about tumoriginicity. A large body of experimental evidence was then generated that showed, in a robust and consistent fashion, that skeletal myoblasts implanted in post-infarcted myocardium differentiated into myotubes and improved left ventricular function. Although the precise mechanism of action was still elusive, these experimental data paved the way for the early-phase clinical studies, which were then followed by a first wave of randomized controlled trials. Now that the results of these trials are available, one has to admit that, once again in medicine, clinical outcomes have not matched the hopes raised by the animal data. In the MAGIC (Myoblast Autologous Grafting in Ischemic Cardiomyopathy) trial (3), which randomized 97 patients, the primary end points (an improvement in global and regional left ventricular function) were not achieved despite a significant reversal of remodeling in patients receiving the highest dose of myoblasts, compared with that of the placebo-injected group. More recently, a catheter-based study, which has entailed the endoventricular delivery of myoblasts, has also failed to show substantial benefits in the treated cohort (P. Serruys, personal communication, March 2008).

Several factors could have accounted for these disappointing results, including a low rate of initial cell retention, a high rate of subsequent cell death, and the inability of engrafted myoblasts to establish functional electromechanical connections with the host cardiomyocytes (4). However, there is another factor of failure that has been so far poorly investigated: the use of the bulk of *unfractionated* myoblasts, as opposed to a specific subpopulation of these myogenic cells that might feature a greater cardiac repair potential. The major interest of the paper by Okada et al. (5) published in this issue of the *Journal* is to have identified, characterized, and functionally assessed such a population. Indeed, in 2005, Winitsky et al. (6) described, in the mouse muscle, a pool of cells that they named skeletal precursors of cardiomyocytes because of their purported ability to differentiate into cardiomyocytes. That same year, Oshima et al. (7) described a population of *murine* muscle-derived stem cells, which, despite the expression of surface markers different from those of skeletal precursors of cardiomyocytes, were also reported to acquire a cardiac phenotype, partly by fusion with host cardiomyocytes. Of note, in a rat model of myocardial infarction, these cells featured a greater and more sustained engraftment, induced more angiogenesis, and effected greater improvements in left ventricular function than unsorted myoblasts (7). The same group subsequently extended this research by describing, in the *human* muscle, a population of myoendothelial cells (8) defined by a positive staining for the CD56, CD34, and CD144 markers and which was shown to share several similarities with mouse muscle-derived stem cells, including a multidifferentiation potential and an increased resistance to oxidative stress. The latter characteristic was postulated to account for the greater muscle regeneration capacity of these myoendothelial cells compared with myoblasts after transplantation in injured skeletal muscle of severe combined immunodeficiency mice (8).

The study by Okada et al. (5) extends these data and strengthens their potential therapeutic interest by showing that this population of myoendothelial cells also displays a cardiac repair capacity. The authors used a mouse model of acute infarction and a comprehensive combinatorial ap-

*Editorials published in the *Journal of the American College of Cardiology* reflect the views of the authors and do not necessarily represent the views of *JACC* or the American College of Cardiology.

From the Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Department of Cardiovascular Surgery; Université Paris Descartes, Faculté de Médecine; and the INSERM U 633, Paris, France.

proach, which allowed them to demonstrates that after 6 weeks the myoendothelial cells provide a better recovery than unsorted myoblasts or sorted populations of either myogenic ($CD56^+CD34^-CD144^-$) or endothelial cells ($CD56^-CD34^+CD144^+$). This conclusion was based on a greater preservation of left ventricular systolic function (but without limitation of remodeling), less scar formation, increased cell engraftment, angiogenesis, and endogenous cardiomyocyte proliferation and decreased early post-infarct apoptosis compared with cells defined by a merely myogenic or endothelial phenotype. These benefits are significant, but those pertaining to function should be interpreted cautiously in that baseline measurements were apparently not performed so that evidence for a strict comparability of initial infarct sizes is lacking; under these conditions, the interpretation of between-group post-transplantation outcomes is clearly less straightforward. Overall, however, the protective effects of the myoendothelial cells look convincing and seem to be primarily related to paracrine effects mediated, in part, by vascular endothelial growth factor whose secretion was expectedly shown to be up-regulated under hypoxic conditions.

Because the clinical results of the early-phase cell therapy trials have not matched the initially high level of expectations (this is not specific for myoblasts in heart failure but also extends to bone marrow cells in acute myocardial infarction), a part of the medical community is now wrongly inclined to step back from this mode of therapy. The fact is that the field is still at an early stage and that we are struggling to find the right cells and the right mode of delivering them in the right patient population. To some extent, the situation is not so different from that of the first heart transplantations, which were associated with a distressingly dismal prognosis. If basic scientists and clinicians had then stopped being committed to pursue this approach, one would never have found the immunosuppressive drugs that have strikingly improved transplantation outcomes. Similarly, we now have to use the existing database on cardiac cell therapy as a building block to move the field forward. The carefully designed and executed study by Okada et al. (5) could contribute to this endeavor by raising the possibility that skeletal myoblast transplantation might be optimized by extracting from the heterogeneous pool of myogenic cells a discrete fraction that combines a dual potential for myogenesis and angiogenesis and can thus contribute to its self-survival. However, the clinical relevance of these findings requires that at least 3 main issues be addressed.

The first is related to scale-up. This is particularly critical if one takes into account that this myoendothelial cell population only represented a tiny fraction of the whole muscular biopsy (1.8%). This number might be even smaller if cells were harvested from older donors (a likely situation in cardiac cell therapy), and, furthermore, it likely decreases during cell processing because of the phenotypic changes occurring over the course of expansion. From this standpoint, it is noteworthy that these cells are reported to have retained their endothelial phenotype, which contrasts with the common observation that the $CD34^+$ antigen usually disappears after several passages. Furthermore, in the current study, Okada et al. (5) injected cells in the acutely infarcted mouse heart. The time required for growing these cells (a few weeks) is clearly not compatible with an emergency setting, and, in clinical practice, these cells look better suited for a more chronic use in patients with heart failure. In this case, the cardiomyocyte deficit has been estimated in the range of 1 billion (9), and it is thus critical to validate, in a large animal model more closely mimicking the human situation, that this $CD34^+CD56^+CD144^+$ population can be expanded up to the high target cell numbers required for adequate cardiac repair. It is equally important to ensure that the local signals that may help drive their fate in situ, and which were apparently present in the acutely injured myocardium, will still be operative once scarring has occurred.

The second issue is related to the functional integration of the cellular graft. The data reported in this study clearly indicate that these myoendothelial cells fail to differentiate into cardiomyocytes. While this does not preclude cardioprotective effects mediated by the paracrine activation of host-associated cytoprotective pathways, these effects might not be sufficient to translate into clinically meaningful improvements in patient outcomes, which likely requires the provision of new donor-derived cardiomyocytes for replacing the dead ones. From this standpoint, it is noteworthy that 8 skeletal muscle-resident stem cell populations have now been described (10). These populations differ by several aspects (origin, method of isolation, growth conditions, differentiation potential), but, importantly, only 2 of them (both of murine origin) have been shown to display a cardiac differentiation potential, which was actually demonstrated in vivo in only 1 case (6). It is, therefore, likely that regardless of their paracrinally mediated cardioprotective effects, the human skeletal muscle-derived cells cannot achieve the ultimate objective of creating new myocardium, which should stimulate research aimed at identifying alternate sources of clinically usable cardiac progenitors (11).

The third issue is related to the potential arrhythmogenic potential of these myoendothelial cells. In the MAGIC trial where all patients were instrumented with an internal cardioverter-defibrillator, there was no significant difference, at the 6-month study point, in the number of arrhythmic events between myoblast-treated patients and those injected with a placebo solution (3). However, a greater propensity for arrhythmias during the early post-operative period cannot still be completely eliminated and is usually attributed to the electrical insulation of the cell clusters that may create conduction blocks setting the stage for re-entries (12). This, in turn, is thought to reflect the failure of myoblasts to express connexin-43. As the $CD34^+CD56^+CD144^+$ population described by Okada et al. (5) does not express this gap junction protein, its potential proarrhythmic risk cannot be eliminated and

should be more thoroughly assessed in clinically relevant large animal models.

It is still too early to know whether the myoendothelial cell population described in this study will ever come to clinical practice. The merit of this paper, however, is to highlight that our use of stem cells has, so far, been rather crude and that refining cell identification and sorting could be a fruitful approach for upgrading the clinical outcomes of cell transplantation.

Reprint requests and correspondence: Dr. Philippe Menasché, Hôpital Européen Georges Pompidou, Cardiovascular Surgery, 20 rue Leblanc, Paris 75015, France. E-mail: philippe.menasche@egp.aphp.fr.

REFERENCES

1. Leor J, Patterson M, Quinones MJ, Kedes LH, Kloner RA. Transplantation of fetal myocardial tissue into the infarcted myocardium of rat. A potential method for repair of infarcted myocardium? Circulation 1996;94:II332–6.
2. Pouly J, Bruneval P, Mandet C, et al. Cardiac stem cells in the real world. J Thorac Cardiovasc Surg 2008;135:673–8.
3. Menasché P, Alfieri O, Janssens S, et al. The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. Circulation 2008;117:1189–200.
4. Reinecke H, Poppa V, Murry CE. Skeletal muscle stem cells do not transdifferentiate into cardiomyocytes after cardiac grafting. J Mol Cell Cardiol 2002;34:241–9.
5. Okada M, Payne TR, Zheng B, et al. Myogenic endothelial cells purified from human skeletal muscle improve cardiac function after transplantation into infarcted myocardium. J Am Coll Cardiol 2008;52:1869–80.
6. Winitsky SO, Gopal TV, Hassanzadeh S, et al. Adult murine skeletal muscle contains cells that can differentiate into beating cardiomyocytes in vitro. PLoS Biol 2005;3:e87.
7. Oshima H, Payne TR, Urish KL, et al. Differential myocardial infarct repair with muscle stem cells compared to myoblasts. Mol Ther 2005;12:1130–41.
8. Zheng B, Cao B, Crisan M, et al. Prospective identification of myogenic endothelial cells in human skeletal muscle. Nat Biotechnol 2007;25:1025–34.
9. Murry CE, Reinecke H, Pabon LM. Regeneration gaps: observations on stem cells and cardiac repair. J Am Coll Cardiol 2006;47:1777–85.
10. Arsic N, Mamaeva D, Lamb NJ, Fernandez A. Muscle-derived stem cells isolated as non-adherent population give rise to cardiac, skeletal muscle and neural lineages. Exp Cell Res 2008;314:1266–80.
11. Tomescot A, Leschik J, Bellamy V, et al. Differentiation in vivo of cardiac committed human embryonic stem cells in postmyocardial infarcted rats. Stem Cells 2007;25:2200–5.
12. Abraham MR, Henrikson CA, Tung L, et al. Antiarrhythmic engineering of skeletal myoblasts for cardiac transplantation. Circ Res 2005;97:159–67.

Key Words: stem cells ■ skeletal myoblasts ■ heart failure ■ transplantation ■ myocardial infarction.