Stem Cell Therapy for Heart Failure
Are Arrhythmias a Real Safety Concern?

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Abstract—So far, the major safety issue raised by the use of stem cells for cardiac repair has been the occurrence of ventricular arrhythmias, particularly after skeletal myoblast transplantation. Although one cannot refute a potential intrinsic arrhythmogenicity of stem cells, primarily related to their common lack of electromechanical integration into the recipient myocardium, it is also important to recognize that patients eligible for cell replacement therapy are prone to develop arrhythmias because of their underlying ischemic heart disease. Another confounding factor is the method used for the intramyocardial delivery of the cells, which can cause enough inflammatory tissue damage to further increase ventricular irritability on top of an already high baseline level. Thus any strategy designed to minimize the risk of stem cell–associated ventricular arrhythmias should take into account, besides the cell-specific ability to appropriately couple with host cardiomyocytes, the method of cell transfer and the nature of the myocardial environment targeted for cell engraftment. A more accurate characterization of the baseline risk of arrhythmias in these patients would thus be helpful for better assessing the respective contribution of the donor cells and the host myocardium to these complications. The risk-to-benefit ratio of stem cell therapy will finally have to be revisited in light of the fact that because this baseline risk is usually high, most of these patients will in any way be fitted with an implantable defibrillator. (Circulation. 2009;119:2735-2740.)

Key Words: arrhythmia ■ cells ■ heart failure ■ myocardial infarction ■ stem cells

The clinical acceptance of any new therapy is largely based on the risk-to-benefit ratio. In the case of stem cell treatment for chronic heart failure, the clinical results have so far been marginally successful, when not negative, whereas the concern that intramyocardial delivery of cells, particularly skeletal myoblasts, could cause potentially life-threatening ventricular arrhythmias has been repeatedly raised. However, in this specific context of heart failure, the legitimate emphasis on this safety issue may have led to somewhat underscore the fact that most of the patients eligible for cell replacement therapy are at high risk of arrhythmias because of their underlying ischemic heart disease to such an extent that they are now increasingly fitted with internal cardioverter-defibrillators (ICDs).1 The objective of the present article is thus to highlight the fact that the proarrhythmic risk of stem cell therapy may not be entirely related to the cells themselves and, at least on the basis of in vitro culture systems or rodent experiments, is reported to be more prominent after skeletal myoblast than after bone marrow cell transplantation.

Skeletal Myoblasts
Heterogeneity of action potential duration increases the incidence of arrhythmias and is expected to occur when skeletal myoblasts are implanted into infarcted myocardium because of the differences in action potential duration between these cells and host cardiomyocytes,2 in addition to the related risk of reentry. Furthermore, skeletal myoblasts downregulate the expression of connexin 43 as they differentiate into myotubes,3 which accounts for the lack of electrical coupling between the grafted cell clusters and the surrounding host myocardium.4 The role of this insulation in the genesis of posttransplantation arrhythmias is illustrated by the finding that myoblast-associated reentrant arrhythmias can be significantly reduced by genetically making these cells and, at least on the basis of in vitro culture systems or rodent experiments, is reported to be more prominent after skeletal myoblast than after bone marrow cell transplantation.

Summary of Experimental Data on Stem Cell–Induced Arrhythmias

Basically, there are 3 major mechanisms that can cause arrhythmias: reentry, automaticity, and triggered activity. Several studies have reported that each of them can be exhibited by stem cells. This proarrhythmic risk has differed with the nature of these cells and, at least on the basis of in vitro culture systems or rodent experiments, is reported to be more prominent after skeletal myoblast than after bone marrow cell transplantation.
lower incidence of arrhythmias after transplantation of these cells whose engraftment rates are low, regardless of whether their delivery is intramyocardial or intracoronary. Transplanted myogenic cells also display automaticity. Even though differentiated myotubes are not connected to the neighboring cardiomyocytes by gap junctions, their intrinsic contractile activity could increase cardiac excitability through electrotonic interactions. Thus in a study in which rat myoblast and cardiomyocyte sheets fabricated by using temperature-responsive culture dishes were overlaid and cocultured, automaticity was displayed by 29% of the myotubes in the myoblast sheet and, in 20% of these cases, resulted in a fibrillation-like stretch-mediated activity in the cocultured cardiomyocyte sheet.

Finally, the spontaneous electrical activity of engrafted myoblasts may provoke after-depolarizations and thus induce ventricular arrhythmias by triggered activity. Coculture experiments have also shown that, possibly because of a paracrinally induced myocyte hypertrophy, cardiac repolarization may be delayed, which has also been associated with triggered activity. However, such a hypertrophy has not been found consistently. Overall, these coculture experiments have been helpful in deciphering the potential mechanisms underlying arrhythmias, but one of their limitations is the use of a 2-dimensional monolayer. The incidence of arrhythmias might actually be lower in the 3-dimensional heart because of the greater number of pathways available for propagation of electrical influxes around the localized barriers formed by engrafted cells interspersed around host cardiomyocytes.

The translation of these in vitro data to the in vivo situation has primarily entailed the use of rodents. In a rat model of myocardial infarction, Fernandes et al used programmed electrical stimulation and showed an increased inducibility of ventricular arrhythmias in myoblast-injected hearts compared with controls. Of note, however, an implantable telemetry system failed to document a difference in the number of arrhythmic episodes between myoblast-injected rats and those receiving control medium. In that same study, injections of bone marrow–derived autologous cells did not result in an increased inducibility of ventricular arrhythmias compared with controls, thereby leading the authors to conclude that myoblasts exhibited a specific arrhythmogenic risk. This conclusion has then been endorsed by the study of Coppen et al, which related the late-phase arrhythmogenicity after myoblast transplantation to a persistent down-regulation of connection 43, possibly mediated by a myoblast-induced upregulation of interleukin-1β. The similar observations of Mills and coworkers that myoblasts cause a greater arrhythmia inducibility than mesenchymal stem cells are more difficult to interpret in that the former cells were injected directly into the myocardium, whereas the latter were delivered intravenously. As discussed below, this difference in the cell delivery routes could have been a confounding factor. In fact, in the study by Roell and associates, the ventricular tachycardia (VT) inducibility did not differ between skeletal myoblasts and bone marrow cells when the 2 cell types were intramyocardially injected in acutely cryolesioned mouse hearts.

Although these rodent data have had the merit of drawing attention on possible life-threatening complications that, in several cases, led to changes in clinical protocols (and primarily to a broader use of ICDs), they are also limited by the fact that the electrophysiological properties of rat or mouse hearts markedly differ from those of humans. This might explain why the conclusions were less clear-cut in a canine heart model, where optimal mapping of left ventricular wedge preparations showed that abnormal impulse propagation and arrhythmia inducibility were primarily related to the presence of infarcted tissue and were actually not worsened by additional myoblast injections.

Mesenchymal Stem Cells

In vitro experiments entailing cocultures of human mesenchymal stem cells (MSC) and neonatal rat ventricular cardiomyocytes have also reported a decreased conduction velocity compared with controls, along with a high percentage of reentries when mixtures of the 2 cell populations contained more than 10% MSCs. The mechanism underlying these arrhythmias may be different from that seen with myoblasts in that MSCs express functional connexin 43–supported gap junctions, thereby leading to postulate that arrhythmias were rather caused by the coupling of cardiomyocytes with inexcitable MSCs. Furthermore, in a swine model of myocardial infarction, injection of MSCs has been shown to stimulate sympathetic innervation, which could increase the risk of arrhythmias.

Clinical Data

We first raised the red flag when 4 patients of the 10 included in our phase 1 trial of myoblast transplantation demonstrated early postoperative ventricular arrhythmias. The other 3 early-phase studies of adjunct-to-coronary artery bypass myoblast injections reported variable results ranging from no to few episodes of ventricular arrhythmias. Likewise, the few catheter-based trials have yielded variable data. Thus in one study entailing the endoventricular delivery of skeletal myoblasts, there were 2 early deaths from arrhythmias and another case of severe nonlethal arrhythmia (of a 14-patient cohort). The study protocol was then changed to include the systematic implantation of an ICD which, at the end of the 4-year follow-up, was found to have fired much more frequently in the cell-treated patients than in the nonrandomized controls. It remains, however, difficult to determine, in this particular study, the respective contribution of the myogenic nature of the cells, their mode of processing, or the endoventricular procedure itself. Conversely, in another study in which myoblasts were delivered by a coronary sinus catheter, there was a single case of VT, resulting in ICD shots in the only patient who was not prophylactically treated with amiodarone.

Because the lack of control groups in all these series made it impossible to draw meaningful conclusions about a possible cause-and-effect relationship between myoblast grafting and ventricular electrical irritability, we tried to clarify this issue by implanting an ICD in every patient included in the randomized controlled Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial, both for safety reasons and also for using ICD read-outs as an objective
means of comparing the prevalence of arrhythmias between the patients who underwent myoblast transplantation and those injected with a placebo medium. All ICD data were then reviewed by an independent expert electrophysiologist blinded to the treatment group. At the 6-month study point, the proportion of patients who had experienced arrhythmias was not significantly different between the 2 myoblast-treated groups (which differed by the number of injected cells: 400 or 800 million) and the placebo-injected group, whereas amiodarone therapy was evenly distributed across the groups. The hazard ratio data did not support either a dose-response increase in arrhythmic episodes in contrast to what would have been predicted from the coculture experiments. Analysis of the time course of events revealed that arrhythmias were primarily clustered in the early postoperative period after myoblast injections, whereas they were more evenly distributed over time in the placebo arm of the trial. There was not a single death that could be attributed to an arrhythmic episode. Although reassuring, these data should be cautiously interpreted because of the small sample sizes (33, 30, and 34 patients in the low cell dose, high cell dose, and placebo groups, respectively). Of note, in the catheter-based study reported by Veltman and coworkers, 24-hour Holter monitoring at the end of the 4-year follow-up similarly showed that equal numbers (roughly one half) of patients in the treated as well as in the control group experienced episodes of nonsustained VT. Put together, these data confirm the ventricular irritability that characterizes these high-risk patients and justify the broad indications for fitting them with ICDs, irrespective of any cell therapy.

By comparison, the clinical results of bone marrow cell transplantation are more straightforward. Most of the studies have entailed direct intracoronary infusions of mononuclear cells in patients with acute myocardial infarction, and it is clear that they have reported an excellent safety record, particularly with regard to arrhythmias, which may be due to the intrinsically low arrhythmogenicity of these cells, but also to the route of delivery and the clinical setting. The importance of the latter 2 factors is illustrated by the fact that intramyocardial injections of bone marrow cells, either in conjunction with coronary artery bypass or isolated in patients with ischemic or nonischemic chronic heart failure, respectively, have also raised arrhythmia-associated safety concerns. As for intravenous injections of MSC, their safety can be predicted from the intramyocardial dispersion of the few cells that home to the target myocardial areas, thereby resulting in a MSC:cardiomyocyte ratio too low for being proarrhythmic.

Thus there is currently compelling evidence that stem cells, and in particular, skeletal myoblasts, may cause arrhythmias or at least increase the potential for these complications; it is also important to recognize that cells have usually been injected directly into chronically scarred myocardium, mostly during surgical procedures. The role of the delivery technique and of the host tissue in the genesis of arrhythmias associated with stem cell therapy is thus important to assess for developing appropriate protective strategies.

Stem Cell Therapy–Dependent but Cell-Independent Contributing Factors

Mode of Cell Delivery

The role of this factor is clearly apparent from the study by Fukushima and associates. Three weeks after a coronary artery ligation–induced myocardial infarction, these authors injected male bone marrow–derived mononuclear cells from green fluorescent protein-transgenic male rats into wild-type female rats through either a direct intramyocardial or a retrograde intracoronary route. Twenty days after ligation, left ventricular function improved to a similar extent regardless of the delivery route, and graft survival was likewise similar in the 2 groups until 84 days after cell injections. Conversely, continuous monitoring of the ECG initially by telemetry (until 7 days after cell injections) and then by 24-hour recordings at different time points demonstrated different patterns. Thus, more than 70% of rats in the intramyocardially injected group showed multiform or consecutive ventricular premature complexes between 1 and 7 days after injections, whereas retrograde delivery of the same cells or intramyocardial injections of saline resulted in much fewer arrhythmias. Furthermore, the proportion of rats demonstrating VT increased from 14% at day 1 after transplantation to 43% after 7 days in rats receiving direct intramyocardial injections, whereas the other 2 groups did not show VT throughout the study period. These findings were associated with distinct histological patterns. Thus, intramyocardial injections resulted in clusters containing both donor-derived cells and host-derived inflammatory cells in the infarct border zone, whereas the retrograde delivery group yielded a more homogeneous donor cell dissemination and less inflammatory damage. It is therefore sound to postulate that the combination of cell clusters behaving as physical barriers to electrical impulse propagation and of cytokines released by inflammatory cells invading the needle puncture sites contribute to set the stage for arrhythmias. A similar benefit of the intravascular retrograde approach over direct intramyocardial injections for reducing early-phase arrhythmias in the rat model was recently reported by the same group with the use of skeletal myoblasts. The role of local tissue injury in the pathogenesis of stem cell–related arrhythmias is further supported by the data of Roell and coworkers, showing, in the mouse model of acute cryoinjury, that the VT inducibility did not significantly differ between animals injected intramyocardially with a control medium and those receiving skeletal myoblasts, bone marrow cells, or fibroblasts. Put together, these data could explain why, at the opposite, the intravascular route of cell administration, which avoids direct tissue injury and is associated with low engraftment rates, has usually not raised arrhythmia-related safety concerns in experimental studies or in the few clinical trials of intracoronary bone marrow cell infusions in patients with heart failure. Besides the route for cell delivery, the volume of the injectate per site could also contribute to arrhythmias through an increased myocardial damage, as suggested by the trend toward higher creatine kinase-MB values with increasing total volume injected during endoventricular myocardial cell injections. Put together, these factors probably account for
the large variability in the prevalence of arrhythmias reported across the different clinical series.

**Nature of the Underlying Substrate**

One of the major entry criteria for patients with heart failure enrolled in cell therapy trials is an antecedent myocardial infarction, which actually puts them, from the onset, at high risk of arrhythmias. Heart failure dramatically increases the risk of sudden death, which is 6 to 9 times that of the general population.35 and these data actually account for the survival benefit provided by ICDs in patients with reduced left ventricular ejection fraction after myocardial infarction.1 In practice, patients eligible for cell therapy have usually been selected on the basis of this marker. However, cardiac magnetic resonance has shown that infarct mass and surface area were better predictors of inducibility than ejection fraction.36 Likewise, enhanced border zone function has shown positive correlation with VT inducibility in patients with postinfarction left ventricular dysfunction.37 Another possible confounder is the pattern of perfusion, as absent or poor anterograde or collateral filling of the infarct-related artery seems to be a powerful predictor of susceptibility to ventricular arrhythmias.38 Of note, none of the patients in the MAGIC trial25 underwent revascularization of the cell-treated segments, which could have increased the risk of arrhythmia, as suggested by the observation that, in the trial conducted by Gavira and associates20 in which myoblast-transplanted areas were bypassed, there were no report of postoperative arrhythmias. Finally, more recent studies using contrast-enhanced magnetic resonance imaging have shown that the extent of myocardial tissue heterogeneity in the infarct border zone is correlated with ventricular irritability.39 If one now looks at the protocol of the clinical cell therapy trials reported thus far, it becomes clear that virtually none have incorporated any of the above-mentioned methods for assessing ventricular irritability. If one now looks at the protocol of the clinical cell therapy trials reported thus far, it becomes clear that virtually none have incorporated any of the above-mentioned methods for assessing ventricular irritability. Thus, in the absence of a reliable evaluation of the baseline risk of arrhythmias, it remains difficult to determine to what extent the subsequent stem cell therapy has substantially increased this risk.

These considerations about the role of the target tissue are important because the proarrhythmic risk of myoblast transplantation has been linked to the site of the injections, with those performed in the core of the scar being potentially less arrhythmogenic than those lining the border zone.40 Intraoperatively, infarct areas usually feature a patchy pattern with an intermingling of discrete areas of near-normal myocardium with foci of fibrotic scar. Because cell targeting is still not completely accurate, it is likely that cells are randomly delivered in varying admixtures of nonviable and viable tissue. Conceivably, cells that happen to be injected in myocardial tissue spots where conduction is already slow could further facilitate the development of reentry arrhythmias.

Of note, the role of the underlying substrate in triggering arrhythmias independently of the cell type has also been reported in the different setting of experimental doxorubicin-induced heart failure, where abnormal ventricular conduction properties were similarly worsened by percutaneously delivered stem cells, regardless of whether they were derived from bone marrow or skeletal muscle.41

**Possible Strategies for Preventing Stem Cell Therapy–Associated Arrhythmias**

Because one cannot realistically hope to change the nature of the target tissue where stem cells are implanted, strategies designed to prevent or limit arrhythmias related to the procedure should rather focus on changes in the gene expression of current cell types, use of alternate less arrhythmogenic cell types, or improved methods of cell delivery.

**Genetic Engineering**

In line with the assumption that gap junction coupling was a critical determinant of electrical stability, studies using both cell culture systems5 and rodent models6 have shown that overexpression of connexin 43 in genetically engineered skeletal myoblasts successfully decreased the ventricular susceptibility to arrhythmias. This approach is further rationalized by the fact that remodeling of connexin expression and gap junction organization are consistent features of human heart disease where there is an increased risk of arrhythmias. The clinical translation of this approach is, however, plagued by several hurdles. First, it would need to be demonstrated, in a clinically relevant large animal model, that the expression of connexin 43 is distributed in such a homogeneous way that the intercellular current flow is streamlined, thereby reducing the likelihood of conduction blocks. One would then have to assess the consequences of electrically coupling cells with different action potential properties. The possible impact of connexin overexpression on the differentiation pattern of myoblasts is another unknown factor. Finally, this type of approach would raise the general safety issues associated with gene therapy.

**Alternate Cell Types**

Even though bone marrow cells seem less arrhythmogenic than skeletal myoblasts, their implantation could nevertheless produce local delays in propagation because electrically coupled but inexcitable cells interspersed with normal host cardiomyocytes may behave as current sinks.43 Thus from an electrical viewpoint, neither skeletal myoblasts nor bone marrow cells look like suitable candidates, as cells with poor coupling properties or inexcitability may generate a mosaic pattern of cell-to-cell coupling, favoring the onset of arrhythmias.44 Conversely, a functional integration of the grafted cells such that they can safely and efficiently contribute to increase pump function requires that their phenotype supports electrical propagation, electromechanical coupling, and contraction.45 There is compelling evidence that cells recapitulating the cardiogenic differentiation program are the best suited for meeting this objective, as demonstrated by the finding that transplanted fetal cardiomyocytes exhibit calcium transients synchronous with those of neighboring host cardiomyocytes and have similar kinetics.46 In practice, cardiac progenitor cells derived in vitro from pluripotent embryonic stem cells look particularly appealing because their partial state of differentiation at the time of implantation should allow local cues to complete driving their fate toward a cardiomyocyte-like phenotype,47 including the ability to functionally couple with host cardiomyocytes.48,49 This hypothesis is strongly supported by the finding that conduction velocity within an infarct area was found to be increased more
than 4-fold in mice injected with embryonic cardiomyocytes compared with sham animals. These data are consistent with an increase in intercellular coupling and were actually associated with a reduction in arrhythmogenic events such as focal activity and conduction blocks. There is no doubt that, given the ethical and immunologic issues associated with embryonic stem cell–derived progenitors, reprogrammed adult autologous somatic cells (induced pluripotent cells) are particularly appealing, but a clinical use of these cells first requires a more thorough assessment of the efficiency, safety, and efficacy of the reprogramming process.

Improved Methods of Cell Delivery

Given the above-mentioned tissue damage resulting from multiple needle injections and its potential contribution to ventricular arrhythmias, it may be worth looking at alternative methods of cell transfer based on the epicardial deposition of cell-seeded patches or scaffold-free cell sheets prepared onto temperature-sensitive culture plates. With the caveat that these approaches may be restricted to open-chest procedures, they could have the advantage not only to improve graft viability through maintenance of intercellular connections and survival signals originating from the extracellular matrix, but also to reduce the risk of arrhythmias through appropriate coupling of cells between them within the sheet and with those of the underlying host myocardium.

Future clinical trials should correct for the baseline arrhythmic risk and thus allow to more clearly identify the risk directly attributable to stem cell therapy, taking into account not only the nature of the cells, but also the myocardial microenvironment and the extent of inflammatory damage triggered by cell transfer. Indeed, assuming that most of these patients will be increasingly protected from life-threatening arrhythmias by their ICD, the problem may no longer be to assess to what extent stem cells pose an additional risk of arrhythmias on top of that as a result of the causal ischemic coronary artery disease, but rather whether they provide a true improvement in surrogate markers of left ventricular function and in clinically meaningful patient outcomes. One has to admit that, so far, available studies have not shown that the benefit of myoblast transplantation outweighs its risk of increased arrhythmogenicity, and the fewer trials of bone marrow cells in the context of heart failure, although possibly safer, have not provided either hints of efficacy. Consequently, the goal of replacing dead cardiomyocytes with new, functionally integrated cells should be actively pursued with the hope that, at the end, such a strategy of cell replacement therapy may improve function to such an extent that it can offset the putative cell-associated arrhythmic risk—and maybe even decrease it.

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Disclosures

None.

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