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EDITORIAL COMMENT

Stem Cell Therapy for Myocardial Repair

Is it Arrhythmogenic?*

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Transplantation of both skeletal myoblasts and stem cells into the region of infarcted myocardium results in improved myocardial function in both the murine and porcine infarct models. Intravenous injection of stem cells and bone marrow stimulating cytokines also improves cardiac function (1–4). The optimal cell type and dose, delivery route, delivery catheter, and the timing of cell injection are still being defined. In order for cell therapy to be widely clinically applicable, the optimal cell has to be compatible both mechanically and electrically with the host myocardium. In this issue of the *Journal*, Smits et al. (5) extend the

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observations to humans, confirming the previous observations of Menasche et al. (6,7) that myoblast transplantation improves global and regional left ventricular function late after myocardial infarction. This procedure has clinical application because cells could be delivered by transendocardial injection in the catheterization laboratory.

Nevertheless, the study also exposes a potential serious limitation of both myoblast and stem cell therapy. In the current study (5), one of the five patients had sustained episodes of ventricular tachycardia and required implantable cardioverter-defibrillator (ICD) placement. The investigators also describe a subsequent unpublished experience of two sudden deaths and three serious ventricular arrhythmias in eight additional patients. These data seem to correspond to the Menasche et al. (6,7) experience in which 4 of 10 patients required ICD implantation for ventricular arrhythmias after open chest autologous myoblast transplantation. Although, it remains possible that these arrhythmias reflect the natural history of myocardial infarction rather than the introduction of the new cells, it seems clear that we must consider the potential mechanisms of arrhythmia and strategies to control or eliminate them.

Proarrhythmia after stem cell therapy might be attributed to one or more of the following reasons: 1) heterogeneity of action potentials between the native and the transplanted stem cells; 2) intrinsic arrhythmic potential of injected cells; 3) increased nerve sprouting induced by stem cell injection; and 4) local injury or edema induced by intramyocardial injection.

ELECTROPHYSIOLOGIC HETEROGENEITY

Normal cardiac cells exhibit significant transmural heterogeneity of action potential duration (8). A delicate balance has to be maintained to prevent arrhythmia. When abnormal shortening or prolongation of action potential duration in some, but not all, of the myocardial cells, disturbs this balance, the incidence of arrhythmia increases. Brugada syndrome and long QT syndrome are two examples of increased arrhythmogenesis due to increased transmural dispersion of repolarization (9). In diseased ventricles, the electrophysiologic remodeling alters the ion channel activity (10), which can increase the dispersion of repolarization even before stem cell transplantation. The transplanted cells also might further increase the electrophysiologic heterogeneity.

Leobon et al. (11) used intracellular recordings coupled with video and fluorescence microscopy to study the contractile and intrinsic membrane properties of labeled myoblasts, transplanted into infarcted rat myocardium. The grafted myoblasts differentiated into hyperexcitable myotubes with a contractile activity fully independent of neighboring cardiomyocytes. No structural connection was observed between myotubes or between myotubes and host cardiomyocytes when Alexa Fluor (Molecular Probes), a connexin permeant dye, was injected in myotubes. In contrast, dye coupling was extensively observed between cardiomyocytes. The action potentials evoked intracellularly in myotubes were accompanied by myotube contractions, but these contractions did not spread to neighboring cardiomyocytes. Electrical stimulation of myotubes evoked a slow voltage-dependent discharge with superimposed bursts of action potentials that were similar to thalamic neuron discharges rather than cardiomyocyte activity. These multiple action potentials clearly carry the potential for inducing cardiac hyperexcitability, even in the absence of direct electromechanical coupling, through electrotonic interaction.

This mechanism may be specific for transplanted myoblasts, because embryonic stem cells have been reported to differentiate into a spontaneously contracting functional syncytium with gap junctions distributed along the cell borders (12). Microelectrode array mapping of this early-stage cardiac tissue demonstrated synchronized action potential propagation with stable focal activation and conduction properties. Nonetheless, the conduction-velocity in the stem cells was less than that of intact human heart. The lower gap junction size and density, the significant presence of connexin 45, and less developed ionic channel machinery in early-stage cardiomyocytes may result in slower conduc-

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Table 1. Arrhythmias After Stem Cell Therapy

Study (Ref.)	Cell Type	Route	n	Death	Arrhythmias	ICD
Menasche et al. (6,7)	Myoblasts	Epicardial	10	0	4 (VT)	4
Smits et al. (5)*	Myoblasts	Endocardial	13	2	4 (VT)	2
Strauer et al. (22)	BM cells	Intracoronary	10	0	0	0
Assmus et al. (23)	BM cells	Intracoronary	19	0	0	0
Stamm et al. (24)	BM cells	Epicardial	6	0	2 (SV)	0
Perin et al. (25)	BM cells	Endocardial	14	1 (SCD)	0	0
Fuchs et al. (26)	BM cells	Endocardial	10	0	0	0
Tse et al. (27)	BM cells	Endocardial	8	0	0	0

References 25, 26, and 27 were studies primarily directed towards angiogenesis rather than myogenesis. *The "n" in the Smits et al. study includes eight patients described in the "Discussion" section who received catheter-based myoblast transplantation in addition to the five patients in the original study.

BM = bone marrow-derived; ICD = implantable cardioverter-defibrillator; SCD = sudden cardiac death; SV = supraventricular arrhythmias; VT = ventricular tachycardia.

tion velocities and a potential substrate for re-entrant arrhythmias.

INTRINSIC ARRHYTHMOGENICITY OF TRANSPLANTED CELLS

Zhang et al. (13) studied the intrinsic arrhythmogenic properties of cardiomyocytes derived from mouse pluripotent embryonic stem cells in vitro using the whole-cell patch-clamp method. Cultured stem cell-derived cardiomyocytes exhibited morphologic heterogeneity of action potentials, many of which had reduced maximum upstroke velocity, prolonged durations, and spontaneous electrical activity in culture. These electrophysiologic changes were accompanied by frequent triggered activity and both early and delayed after depolarization with and without pharmacologic enhancement. Thus, cultured stem cells exhibit all of the three arrhythmogenic mechanisms: 1) re-entry, 2) automaticity, and 3) triggered activity.

NERVE SPROUTING

Sympathetic nerve activation exerts significant effects on electrophysiologic properties such as automaticity, triggered activity, refractoriness, and conduction velocity of myocardial cells (14–16). Consequently, an increased and heterogeneous cardiac innervation might amplify the spatial inhomogeneity of electrophysiologic properties and facilitate the initiation of ventricular arrhythmia (17,18). We have demonstrated that mesenchymal stem cell injection to the swine myocardium induces increased sympathetic nerve density throughout the ventricles (19). Thus, although increased sympathetic innervation could increase contractility and ejection fraction of the treated ventricles, it might also induce ventricular arrhythmias by increasing dispersion of repolarization and triggered arrhythmias (20,21), particularly in a damaged ventricle with abnormal ion channel activity caused by electrical remodeling.

LOCAL INJURY

Most cases of arrhythmias in the early clinical trials have been associated with intramyocardial rather than intracoronary injection. Tissue injury could be responsible for arrhythmogenesis after intramyocardial injection. On the other hand, no increase in ventricular arrhythmias has been reported after therapies such as direct laser transmyocardial revascularization that cause local tissue damage and scarring. Nonetheless, local injection also induces a highly uneven distribution of cells, at least early after injection, which increases electrophysiologic heterogeneity.

The nature of the injected cell may have the most impact on arrhythmogenesis after transplantation. Myoblasts and stem cells differ in their inherent electrophysiologic properties and in their ability to couple electromechanically both among themselves and with host cardiomyocytes. Limited clinical data available thus far (22–27) (Table 1) suggest that arrhythmias are more likely to occur after myoblast than after stem cell transplantation. Finally, limited clinical experience suggests that proarrhythmic effects of cell therapy may be transient. Nonetheless, because the occurrence of cardiac arrhythmia is highly unpredictable, long-term follow-up studies of cell transplant recipients would seem to be essential for understanding the natural course of myoblast and stem cell induced arrhythmogenesis.

Future pre-clinical and clinical studies will determine the most effective and safe cell type for myocardial repair and the clinical significance of cell therapy-induced arrhythmias. In the interim, it is prudent to restrict the use of myoblast transplantation to patients with an implanted cardiac defibrillator. The monitoring function of the ICD can provide investigators with accurate statistics on the incidence, types, and time course of arrhythmias associated with cell transplantation. These data will be critical for evaluation of the arrhythmia risks associated with this new option of treating congestive heart failure. Although stem cell therapy has not been reported to induce early cardiac arrhythmias, investigators need to be aware of the potential risk and include careful long-term follow-up for arrhythmia detection in their clinical trials.

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