Stem cell therapy for cardiac arrhythmias

David Mocini, Furio Colivicchi, Massimo Santini

Division of Cardiology, Cardiovascular Department, San Filippo Neri Hospital, Rome, Italy

Key words:
Arrhythmia; Cells; Pacemakers; Transplantation.

Clinical studies suggest that stem cell transplantation (SCT) is feasible and has the potential for beneficial effects in several cardiac affections, including myocardial infarction and advanced heart failure. However, concern exists about the possible occurrence of serious arrhythmias after SCT, even if such complication has been shown only in case of skeletal myoblast transplantation. SCT might induce arrhythmias by several mechanisms, such as electrotonic stimulation of cardiac cells, electrical heterogeneity of action potentials during stem cell differentiation process, increased nerve sprouting, and local tissue injury induced by intramyocardial injection. As a matter of fact, the use of endothelial progenitor cells from the peripheral blood or of stem cells from bone marrow has not been associated with any significant cardiac rhythm disturbance.

Recently, a new opportunity for SCT has emerged: the development of a biological cardiac pacemaker. Both gene therapy and cell therapy have been used in this new perspective. In fact, at present, the transformation of a normal cardiomyocyte in a pacemaker cell can be obtained in animal models by the injection of a plasmid or virus, incorporating the gene encoding for specific proteins. This procedure transforms cardiomyocytes in transgenic cells that may show an overexpression of β_2-adrenergic receptors, or abnormal membrane ion channels. As an alternative, genetically modified mesenchymal stem cells can be delivered within the heart and engraft to develop a biological pacemaker.

To date, several studies have been performed in different animal models employing both cell and gene therapy. However, complex problems concerning safety and efficacy require a solution before we can move to the step of clinical evaluation in human beings.

(Ital Heart J 2005; 6 (3): 267-271)

Address:
Dr. David Mocini
Via Giuseppe Gioeni, 30
00158 Roma
E-mail: david.mocini@fastwebnet.it

Introduction

Stem cell transplantation (SCT) may provide new and clinically relevant options for the treatment of several cardiovascular affections. In fact, in animal models, stem cells can engraft in areas of myocardial damage and differentiate into cardiomyocytes, thereby improving cardiac function[1-3]. Besides, preliminary experiences have recently been performed in patients with ischemic heart disease[4-16]. In these studies different stem cell sources and different methods of delivery have been used with promising results[4-16].

In the field of cardiac arrhythmias SCT retains particular interest for two opposite reasons: 1) SCT has been associated with an increased incidence of cardiac rhythm disturbances; and 2) SCT could represent a promising new therapeutic option in some specific conditions, such as bradyarrhythmias.

Stem cell therapy and arrhythmias

The arrhythmogenic potential of stem cells emerged for the first time in a report by Zhang et al.[17]. In such study, the arrhythmogenic properties of mouse cardiomyocytes derived from multipotent embryonic stem cells and embryonal carcinoma cells were studied *in vitro*. Overall, the differentiated cardiomyocytes demonstrated a spontaneous electrical activity, a prolonged action potential duration, and an easily inducible triggered activity. All of these electrophysiological characteristics could favor an unanticipated arrhythmogenic activity from any of the three classic mechanisms (reentry, automaticity, or triggered activity).

Subsequently, Menasché et al.[18] reported the feasibility and safety of the intramyocardial injection of skeletal myoblasts during coronary artery bypass grafting (CABG).

This study included 10 patients undergoing CABG, all showing a previous myocardial infarction, and a depressed left ventricular function (ejection fraction < 35%). Skeletal myoblasts were directly injected in areas of infarcted myocardium, which were not amenable to revascularization. During the follow-up period, 4 patients had episodes of sustained ventricular tachycardia and underwent the implanta-

tion of a cardioverter-defibrillator. Similar results were reported by Smits et al.[19], who transendocardially delivered the same cell type into the infarcted myocardium of 5 patients with advanced heart failure.

Actually, when injected in an infarcted area, autologous skeletal myoblasts differentiate into hyperexcitable myotubes with a contractile activity that results to be fully independent of neighboring cardiomyocytes. No structural connection can be observed between myotubes or between myotubes and host cardiomyocytes[20]. Consequently, the arrhythmogenic potential of these cellular structures may derive from at least two separate mechanisms: 1) the possibility of generating electrotonic currents, which in turn may alter the action potential of neighboring host cardiomyocytes, and 2) the formation of segregated areas, possibly supporting reentrant arrhythmias.

Differently from clinical experiences with skeletal myoblasts, no significant arrhythmias have ever been noted in recent studies employing autologous stem cells from either the bone marrow or the peripheral blood[4-16]. Actually, from a merely theoretical point of view, autologous stem cells could also induce arrhythmias through three different mechanisms:

• during the differentiation process toward the final mature phenotype, stem cells may evolve through intermediate stages in which the intrinsic electrophysiological properties of the cell membranes are not stable, thereby possibly facilitating rhythm disturbances[17];

• stem cell engrafting could be associated with an increased and heterogeneous adrenergic cardiac innervation, which might amplify the spatial inhomogeneity of electrophysiological properties and facilitate the initiation of ventricular arrhythmia[21];

• local injury or edema induced by intramyocardial injection may directly favor arrhythmias.

Recently, our group transplanted autologous stem cells deriving from the bone marrow in 18 patients undergoing CABG[22]. In our clinical experience, the incidence of arrhythmias during Holter monitoring was similar in transplanted patients and in matched controls, both in the postoperative period and during a 3-month follow-up (Table I). Moreover, during the follow-up period, also the occurrence of any potentially arrhythmia-related clinical event, such as syncope or palpitations, was similar in the two study groups. Overall, even if the available evidence derives from small series, the arrhythmic risk associated with transplantation of autologous stem cells from the bone marrow and the peripheral blood seems to be rather low.

Practical applications of stem cell therapy for arrhythmias

In the field of arrhythmias, both gene and stem cell therapies represent new and promising strategies for the development of a biological pacemaker[23]. Actually, at present, the implantation of electronic devices is the only effective approach for the treatment of symptomatic bradyarrhythmias, including atrioventricular blocks and sinus node dysfunctions. Besides, effective pacemakers are currently implanted with a low incidence of both short- and long-term complications. However, the ideal device is still to come. In fact, the need of implanting endocardial catheters, which in most cases can no longer be removed, remains a major problem for long-term clinical pacing. Furthermore, cardiac pacing in the pediatric age still needs improvements, as the devices cannot follow the somatic growth of the little patients and have to be changed over the years. These are only two examples of the several limitations of the presently used, implantable antiarrhythmic devices.

Stem cell therapy, either by itself, or associated with gene therapy, may actually provide a significant new opportunity for the solution of such problems. In fact, gene and stem cell therapy are rapidly approaching the development of a biologic pacemaker, which, in the long run, will possibly substitute the currently implanted electronic devices.

Table I. Arrhythmias in patients undergoing coronary artery bypass grafting.

	Stem cell transplant group	Control group	p
In hospital follow-up			
Atrial fibrillation (pts)	6	5	NS
Premature ventricular contractions (no./pt) (median)	513	763	NS
Ventricular couplets (no./pt) (median)	6	3	NS
Non-sustained ventricular tachycardia (pts)	5	6	NS
Sustained ventricular tachycardia (pts)	1	0	NS
Post-discharge follow-up			
Atrial fibrillation (pts)	1	1	
Premature ventricular contractions (no./pt) (median)	276	133	NS
Ventricular couplets (no./pt) (median)	0.5	3	NS
Non-sustained ventricular tachycardia (pts)	1	3	NS
Sustained ventricular tachycardia (pts)	0	0	NS

Pacemaker cells differ from common cardiomyocytes for the presence of a spontaneous depolarization process, which progressively reduces the membrane potential during the diastolic phase of the cardiac cycle. When the reduction reaches a critical threshold value, the sodium channels open and the action potential ensues. The spontaneous diastolic depolarization of pacemaker cells is due to the expression of four genes (HCN 1-4), which code for four specific proteins, providing the presence of an inward current named I_f (funny current). Therefore, the main difference between pacemaker cells and other cardiomyocytes depends only upon which genes are fully expressed. Actually, as the same consideration applies to all specialized cells in every human tissue, the knowledge of the specific signals inducing gene expression may provide the unique opportunity of modifying the cellular differentiation process. However, we are presently unable to identify and use those factors inducing the transcription of selected genes. Accordingly, in order to develop a biological pacemaker, different methods cold be presently considered:

1) selective increase of the cellular response to adrenergic stimulation[24,25],
2) reduction of the inward diastolic I_{k1} rectifying current[26,27],
3) increase in the inward depolarizing current[28,29].

As to the first point, in experimental models, the increase of the cellular response to adrenergic stimulation can be obtained by introducing in myocardial cells a plasmid carrying the gene which codifies for the β_2-adrenergic receptor. This procedure is followed by an upregulation of β_2-adrenergic receptors, which in turn increases the cellular response to the adrenergic stimulation and may induce a spontaneous pacemaker activity. However, such approach has inherent limitations, as it does not provide the cellular membrane with new ion channels, but simply modulates the already existing structures. Moreover, the effect usually has a short duration, owing to the rapid development of a downregulation of the same β_2-adrenergic receptors. Finally, such methodology has not proven clearly effective in any severe bradyarrhythmia.

Moving to the second point, we should underline that the inward diastolic rectifying current is related to different ion currents. The best known of these ion fluxes is the so-called I_{k1}. In animal models, the I_{k1} can be reduced by introducing in the cardiomyocyte a modified, dysfunctional version of Kir2.1, which is the gene codifying for the α subunits of the channel devoted to such ion current. The final product of such process is a structurally abnormal ion channel that is responsible for a reduction of the inward rectifying current I_{k1}. Consequently, during the diastolic phase, the membrane resting potential tends to a progressive reduction, that in turn favors the spontaneous generation of the action potential.

As to the third issue, an increase of the inward depolarizing current can be obtained in transgenic cells, that can be induced to express in their membranes large amounts of channels for the I_f current. This strategy has been realized in animal models by directly delivering the genes that code for the HCN2 channel (hyperpolarization-activated cyclic nucleotide-gated) to selected cardiomyocytes[28,29]. These cells, which were originally devoid of any spontaneous diastolic depolarization, have then been transformed in potential pacemaker cells, effectively generating escape rhythms. To date, this last option seems to represent the most effective opportunity for the realization of the biological pacemaker. However, there are still many obstacles to overcome before such pacemaker constructs become feasible for clinical testing. In fact, uncertainties remain with regard to safety, permanence of expression of the delivered genes, as well as to the level of expression necessary to achieve optimal pacemaker rate.

As a matter of fact, all of the above described strategies can be pursued and potentially realized with one or more of the following methodologies:

1) direct intracardiac delivery of transgenic heterologous embryonic stem cells;
2) direct intracardiac delivery of embryonic stem cells deriving from therapeutic clonation techniques, and consequently showing a genetic structure identical to that of the receiving subject;
3) gene therapy with naked plasmids or viral vectors, allowing the *in situ* transformation of atrial or ventricular cardiomyocytes into pacemaker cells;
4) reinjection of previously taken autologous myocardial cells after specific treatments (i.e. biopsy followed by culture and *in vitro* genetic modification);
5) delivery of autologous stem cells after differentiation in a specific cellular phenotype.

As to the first two points, there are specific limitations. First of all, the Italian legislation does not allow the use of embryos for any purpose but reproduction. Besides, the use of embryonic stem cells is still ethically debated.

From a merely technical point of view, it is now possible to obtain cells showing electrophysiological properties similar to that of pacemaker cells. However, we are presently unable to anticipate the risks potentially associated with the use of stem cells, which are not fully differentiated. In fact, even if in an early phase of their development several cellular lines may show the ability of spontaneously depolarize, we still do not know whether this pacemaker capability will be maintained after an *in vivo* transplantation. Actually, the final phenotype could be devoid of such a property. Finally, we should keep in mind that heterologous stem cells are genetically different from the cells of the receiver, and this may induce troublesome immunologic problems.

The direct injection of a plasmid or virus (adenovirus), incorporating the gene encoding for a specific pre-determined protein, may be performed *in vivo* in

both atrial and ventricular cardiomyocytes. This procedure transforms the host cardiomyocytes in transgenic cells showing an overexpression of some pre-selected protein. Actually, in animal models, this methodology has been employed with different genes:
- the gene encoding for the β_2-adrenergic receptor[24,25],
- the gene encoding for the channel HCN2[28,29],
- the modified Kir2.1 gene[26,27].

The transplantation of genetically modified cells with a pacemaker activity has been performed using stem cells from canine bone marrow[30].

Mesenchymal stem cells are multipotent, having the possibility of differentiating in a number of cell lines, including musculoskeletal and connective tissues. As a matter of fact, these cells have diverse properties that are particularly relevant for us in the development of a biologic pacemaker. In fact, mesenchymal stem cells show a weak I_f current, but their membranes have several ion channels available[31]. Besides, these cells appear to be immunoprivileged, that is they do not elicit any major immune response by the host[32]. Finally, they seem able to develop gap junctions with both other mesenchymal cells and host cardiomyocytes[30,33].

Potapova et al.[30] have studied human mesenchymal cells from the bone marrow, which were modified by the insertion of the gene for HCN2. In these cells, the researchers were able to demonstrate the presence of an ion current with the typical properties of I_f. Moreover, when injected in a canine left ventricle, these transgenic mesenchymal cells were able to establish gap junctions with host cardiomyocytes and gave rise to an escape rhythm after the induction of sinus arrest.

Conclusions

The recent introduction of stem cells promises to revolutionize the way in which medicine is nowadays practiced. SCT may favor angiogenesis and myocardial regeneration, while the potential arrhythmic risk associated with such procedure is certainly low.

The development of a biological pacemaker in animal models is currently under way, while several complex issues have to be addressed before transferring the initial and promising results to human beings. In particular, the safety of the procedures is matter of dispute. In fact, we still do not know whether viral gene transfer, embryonic SCT and mesenchymal stem cell delivery may determine any severe adverse event in adult human patients. Furthermore, presently available gene manipulation techniques still need significant improvements in order to ameliorate both efficacy and efficiency. Given all of these concerns, we think that the endeavor is worth the effort and that in the near future electronic devices will be replaced by biological tools.

References

1. Klug MG, Soonpaa MH, Koh GY, Field LJ. Genetically selected cardiomyocytes from differentiating embryonic stem cells from stable intracardiac grafts. J Clin Invest 1996; 98: 216-24.
2. Orlic D, Kajstura J, Chimenti S, et al. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. Proc Natl Acad Sci USA 2001; 98: 10344-9.
3. Malouf NN, Coleman WB, Grisham JW, et al. Adult-derived stem cells from the liver become myocytes in the heart in vivo. Am J Pathol 2001; 158: 1929-35.
4. Assmus B, Schächinger V, Teupe C, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation 2002; 106: 3009-17.
5. Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 2002; 106: 1913-8.
6. Kang HJ, Kim HS, Zhang SY, et al. Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomised clinical trial. Lancet 2004; 363: 751-6.
7. Britten MB, Abolmaali ND, Assmus B, et al. Infarct remodeling after intracoronary progenitor cell treatment in patients with acute myocardial infarction (TOPCARE-AMI): mechanistic insights from serial contrast-enhanced magnetic resonance imaging. Circulation 2003; 108: 2212-8.
8. Wollert KC, Meyer GP, Lotz J, et al. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. Lancet 2004; 364: 141-8.
9. Perin EC, Dohmann HF, Borojevic R, et al. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. Circulation 2003; 107: 2294-302.
10. Fuchs S, Satler LF, Kornowski R, et al. Catheter-based autologous bone marrow myocardial injection in no-option patients with advanced coronary artery disease: a feasibility study. J Am Coll Cardiol 2003; 41: 1721-4.
11. Tse HF, Kwong YL, Chan JK, Lo G, Ho CL, Lau CP. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. Lancet 2003; 361: 47-9.
12. Stamm C, Westphal B, Kleine HD, et al. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. Lancet 2003; 361: 45-6.
13. Hamano K, Nishida M, Hirata K, et al. Local implantation of autologous bone marrow cells for therapeutic angiogenesis in patients with ischemic heart disease: clinical trial and preliminary results. Jpn Circ J 2001; 65: 845-7.
14. Galinanes M, Loubani M, Davies J, Chin D, Pasi J, Bell PR. Autotransplantation of unmanipulated bone marrow into scarred myocardium is safe and enhances cardiac function in humans. Cell Transplant 2004; 13: 7-13.
15. Li TS, Hamano K, Hirata K, Kobayashi T, Nishida M. The safety and feasibility of the local implantation of autologous bone marrow cells for ischemic heart disease. J Card Surg 2003; 18 (Suppl 2): S69-S75.
16. Ozbaran M, Omay SB, Nalbantgil S, et al. Autologous peripheral stem cell transplantation in patients with congestive heart failure due to ischemic heart disease. Eur J Cardiothorac Surg 2004; 25: 342-51.
17. Zhang YM, Hartzell C, Narlow M, Dudley SC Jr. Stem cell-

derived cardiomyocytes demonstrate arrhythmic potential. Circulation 2002; 106: 1294-9.
18. Menasché P, Hagège AA, Vilquin JT, et al. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. J Am Coll Cardiol 2003; 41: 1078-83.
19. Smits PC, van Geuns RJ, Poldermans D, et al. Catheter-based intramyocardial injection of autologous skeletal myoblasts as a primary treatment of ischemic heart failure: clinical experience with six-month follow-up. J Am Coll Cardiol 2003; 42: 2063-9.
20. Pagani FD, DerSimonian H, Zawadzka A, et al. Autologous skeletal myoblasts transplanted to ischemia-damaged myocardium in humans. Histological analysis of cell survival and differentiation. J Am Coll Cardiol 2003; 41: 879-88.
21. Pak HN, Qayyum M, Kim DT, et al. Mesenchymal stem cell injection induces cardiac nerve sprouting and increased tenascin expression in a swine model of myocardial infarction. J Cardiovasc Electrophysiol 2003; 14: 841-8.
22. Mocini D, Staibano M, Menichella G, et al. Trapianto autologo di cellule staminali da midollo osseo in pazienti sottoposti a by-pass aortocoronarico: fattibilità e sicurezza. (abstr) Ital Heart J 2004; 5 (Suppl 5): 33S.
23. Rosen MR, Brink PR, Cohen IS, Robinson RB. Genes, stem cells and biological pacemakers. Cardiovas Res 2004; 64: 12-23.
24. Edelberg JM, Aird WC, Rosenberg RD. Enhancement of murine cardiac chronotropy by the molecular transfer of the human β_2 adrenergic receptor cDNA. J Clin Invest 1998; 101: 337-43.
25. Edelberg JM, Huang DT, Josephson ME, Rosenberg RD. Molecular enhancement of porcine cardiac chronotropy. Heart 2001; 86: 559-62.
26. Miake J, Marbán E, Nuss HB. Biological pacemaker created by gene transfer. Nature 2002; 419: 132-3.
27. Miake J, Marbán E, Nuss HB. Functional role of inward rectifier current in heart probed by Kir2.1 overexpression and dominant-negative suppression. J Clin Invest 2003; 111: 1529-36.
28. Qu J, Plotnikov AN, Danilo P Jr, et al. Expression and function of a biological pacemaker in canine heart. Circulation 2003; 107: 1106-9.
29. Plotnikov AN, Sosunov EA, Qu J, et al. Biological pacemaker implanted in canine left bundle branch provides ventricular escape rhythms that have physiologically acceptable rates. Circulation 2004; 109: 506-12.
30. Potapova I, Plotnikov A, Lu Z, et al. Human mesenchymal stem cells as a gene delivery system to create cardiac pacemakers. Circ Res 2004; 94: 952-9.
31. Heubach JF, Graf EM, Leutheuser J, et al. Electrophysiological properties of human mesenchymal stem cells. J Physiol 2003; 554 (Part 3): 659-72.
32. Di Nicola M, Carlo-Stella C, Magni M, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 2002; 99: 3838-43.
33. Valiunas V, Doronin S, Valiuniene L, et al. Human mesenchymal stem cells make cardiac connexins and form functional gap junctions. J Physiol 2004; 555 (Part 3): 617-26.