Stem Cells to Regenerate Cardiac Tissue in Heart Failure

Felipe Prósper Cardoso[a], Jesús Herreros González,[b] and Eduardo Alegría Ezquerra[b]

[a]Servicio de Hematología y Área de Terapia Celular, Clínica Universitaria. Universidad de Navarra, Pamplona. [b]Departamento de Cardiología y Cirugía Cardiovascular, Clínica Universitaria, Universidad de Navarra, Pamplona, Spain.

Myocardial regeneration is one of the most promising therapeutic strategies for heart failure patients. Many experimental studies have demonstrated that different types of stem cell can differentiate into myocardial cells and tissues necessary for regeneration of the damaged myocardium, while studies in experimental animals suggest that muscle (myoblast), bone marrow (mesenchymal, endothelial or hematopoietic progenitors) and even heart cells can help to improve heart contractility *in vivo*. These findings have led several groups to undertake studies in patients with myocardial infarction. However, the use of cellular therapy in clinical trials is not without controversy, mainly related with the need for better knowledge before these therapeutic strategies are used in clinical practice. Although significant enhancement of our knowledge of the processes involved is fundamental, we do not consider it unreasonable to initiate clinical trials in which specific questions are posed, whose answers will allow us to make further progress.

Key words: *Heart failure. Stem cells. Myoblasts. Cardiac regeneration. Transdifferentiation.*

Full English text available at: www.revespcardiol.org

Utilización de células madre para la regeneración miocárdica en la insuficiencia cardíaca.

La terapia celular en la reparación miocárdica se vislumbra como una de las estrategias terapéuticas con mayor futuro en el tratamiento de la insuficiencia cardíaca. Numerosos estudios *in vitro* recientes apoyan la potencialidad de distintos tipos de células madre de diferenciarse hacia los tejidos necesarios para regenerar el tejido miocárdico dañado, mientras que estudios en animales de experimentación sugieren que células madre de músculo (mioblastos), médula ósea (progenitores mesenquimales, endoteliales o hematopoyéticos) e incluso del propio corazón pueden contribuir *in vivo* a mejorar la contractilidad cardíaca. Estos trabajos han conducido a que diversos grupos hayan iniciado estudios en pacientes con infarto de miocardio. Sin embargo, la utilización de la terapia celular en ensayos clínicos no está desprovista de controversia, fundamentalmente relacionada con la necesidad de aumentar nuestro conocimiento antes de pasar a la aplicación clínica de estas estrategias terapéuticas. Aunque es fundamental aumentar significativamente el conocimiento de los procesos, no consideramos irrazonable iniciar ensayos clínicos en los que se identifiquen preguntas concretas cuya respuesta nos permita avanzar en esta dirección.

Palabras clave: *Insuficiencia cardíaca. Células madre. Mioblastos. Regeneración cardíaca. Transdiferenciación.*

The use of cells for tissue regeneration or repair is one of the most promising areas in biomedical research. The idea of regenerative medicine based on the use of the patient's own cells is attracting increasing interest.[1] However, only very preliminary trials are in progress and the translation of these strategies into clinical practice is still some way ahead. We present a perspective of the current state of heart cell regenerative therapy based on the recent experience of several groups involved in this field, and identify some of the main issues.

STEM CELLS AND CARDIOMYOCYTES

Different types of stem cells, both in embryonic and adult tissues, are able to proliferate and produce mature functional cells. Embryonic stem cells can

Study partly financed by grants from the Ministerio de Ciencia y Tecnología (SAF 2002-04574-C02), European Funds FEDER (INTERREG IIIA), and the Universidad de Navarra (PIUNA).

Correspondence: Dr. F. Prósper.
Servicio de Hematología y Área de Terapia Celular.
Avda. Pío XII, 36. 31008 Pamplona. Spain.
E-mail: fprosper@unav.es

differentiate into almost any of the more than 200 tissues existing in an adult organism. Within the different organs are pluripotential stem cells able to differentiate into functional tissues, such as liver stem cells (oval cells), nerve stem cells, muscle stem cells, and gastrointestinal or bone marrow stem cells. Although still controversial, evidence suggests that certain adult stem cells are much more pluripotential than originally thought, and may also give rise to most adult tissues.[2-6]

The possibility of obtaining muscle and endothelial tissue from stem cells, together with the high incidence of patients with heart failure, the limited efficacy of medical treatment and the lack of available organs for heart transplants have led to the application of stem cell therapy for the treatment of patients with heart failure, mainly of ischemic origin.[7-11]

Embryonic stem cells

Despite the fact that embryonic stem cells can differentiate into cardiomyocytes and regenerate myocardium,[12] their application has been restricted to experimental animal studies because of their immunogenic potential,[13] the possibility of generating tumors in vivo, their potential to cause arrhythmia,[14] and certain ethical issues.

Cardiac stem cells

As has happened with nerve cells, the traditional view that cardiomyocytes are unable to proliferate has had to be revised as a result of several recent studies, which demonstrate the existence in the human heart of cells with proliferative capacity.[15,16] It is not known whether these cells are indeed present in the heart (cardiac stem cells in the atrium), or whether they derive from other adult tissues such as bone marrow.[17] Although these studies are of great biological interest, they seem unlikely to have important clinical repercussions for patients with myocardial infarction.[18] Nevertheless, the possibility of stimulating the migration and proliferation of stem cells *in vivo* is of great therapeutic interest. Recent studies in animal models given growth factors suggest that it may be possible to stimulate heart regeneration by «mobilizing» these stem cells.[19]

Bone marrow stem cells

Stem cells derived from bone marrow have shown the greatest ability to differentiate into heart muscle fibers or endothelial cells, thereby contributing to angiogenesis or vasculogenesis.[7,20-24] Unlike those studies in which mononuclear bone marrow cells were used,[20] work by Anversa and Orlic's group used selected enriched populations of hematopoietic bone marrow stem cells. They demonstrated that intracardiac injection of Lin– Kit+ cells into the infarction scar in a rat model of infarction induces colonization of the scar by cardiomyocytes and vascular structures derived from the implanted cells.[23] This regeneration was accompanied by improved cardiac function and increased survival of the animals. In addition to hematopoietic stem cells, bone marrow contains endothelial stem cells able to contribute to neo-angiogenesis, favoring myocardial regeneration.[24-26] The systemic administration of these cells in infarction models helped to improve cardiac function[24] as a result of increased vasculogenesis in the infarcted area, reduced cardiomyocyte apoptosis and improved contractility. Endothelial stem cells, however, do not acquire the characteristics of heart muscle. Mesenchymal stem cells from bone marrow are able to differentiate into mesodermal tissues such as osteoblasts, chondrocytes, adipocytes or skeletal muscle.[27] Recent studies even indicate that mesenchymal stem cells are able to differentiate into cardiomyocytes both *in vitro* and *in vivo*.[20,28] Using animal models of infarction, several groups have shown that mesenchymal stem cells injected into the myocardial scar are not only able to engraft but also acquire the characteristics of cardiomyocytes, and more importantly, contribute to improved cardiac function.[20,29] A subpopulation of pluripotential mesenchymal stem cells called MAPC was recently described as able to differentiate into tissues derived from any of the three embryonic layers.[5,30]

Muscle stem cells

In addition to bone marrow or cardiac stem cells, muscle stem cells (satellite cells or skeletal myoblasts), which are precursors of muscle fibers, may also have practical applications as they possess undoubted muscular potential.[31] The possibility of *in vitro* expansion of the number of muscle progenitor cells has made studies in animal models of infarction possible. Implanted myoblasts were able to engraft and terminate the differentiation process into muscle fibers, thereby contributing to improved cardiac function and survival of the animals.[32,33] Myoblasts can be administered not only by intracardiac injection but also percutaneously,[34,35] since they are able to migrate via the microcirculation and integrate into the interstitium. One of the main disadvantages of muscle stem cells is their inability to acquire the necessary characteristics of heart muscle to enable transmission of the electromechanical stimulus, and thereby improve myocardial function.[33,36,37]

Experimental *in vitro* and *in vivo* studies with animals undoubtedly support the existence of stem cells able to contribute to improved cardiac function. However, numerous questions still remain and efforts

must be made to provide answers which justify therapeutic studies of these treatments. Stem cells clearly exist with endothelial, cardiac and muscle potential, but it is necessary to identify these cells and determine which signals are required to develop this potential, as well as the signals required to ensure that their potential is developed in harmony with their surrounding tissues, avoiding loss of functionality.

CLINICAL EXPERIENCE IN REGENERATIVE CARDIAC THERAPY

Despite the many unknowns, results accumulated over recent years from preclinical trials have led to the first clinical trials of the feasibility and safety of cardiac regeneration with stem cells. These trials have often been accompanied by a certain degree of controversy, owing to the opinion of researchers who argue that evidence is insufficient to justify clinical research. Seven clinical trials have so far been published[7-11,38,39] on the use of percutaneous, intracavitary or intramyocardial infusion of bone marrow–derived mononuclear cells and cells enriched in hematopoietic, endothelial or myoblast progenitor cells. The results were monitored by function and imaging techniques such as magnetic resonance imaging, echocardiography or positron emission tomography. Control groups have occasionally been used to compare results between patients either receiving or not receiving cells, but in all cases the patients also received additional therapy.

Menasché et al pioneered the use of skeletal myoblasts and carried out the first implant of autologous myoblasts in a patient with an infarction in June 2000.[40] Their strategy consisted of obtaining a muscle biopsy 2-3 weeks before revascularization surgery in patients with an old infarction and non-viable myocardial tissue in the area. After *in vitro* culture the cells are then implanted by intra-myocardial injection into the peri-infarction area during surgery. Results from the first ten patients who underwent this treatment were recently published.[11] They suggest improved cardiac function with a significant increase in ejection fraction. However, despite the fact that the cells were injected into non-revascularized areas, it is impossible to determine with certainty whether the effect was due to the bypass surgery or the implantation of myoblasts. Notably, four of the ten patients included in the study had ventricular arrhythmia (ventricular tachycardia) which required implantation of a defibrillator. This suggests that treatment with myoblasts may induce arrhythmias.[39]

Other groups have used mononuclear bone marrow cells instead of myoblasts.[8,10] Strauer et al used coronary angiography to inject mononuclear bone marrow cells into patients with acute myocardial infarction at the same time as they inserted a coronary stent, during the immediate post-infarction period.[8] Their control group was composed of 10 patients who underwent the same therapeutic procedure except for cell injection. Ten weeks after treatment the size of the infarction was reduced, and there were increases in ejection fraction, cardiac index and systolic volume in the treatment group compared to the control group. This improvement in ventricular function was attributed to increased myocardial perfusion as measured by radioisotope studies. In the study by Tse et al, 8 patients with severe ischemic heart disease received mononuclear bone marrow cells via intracardiac catheter guided by electromechanical mapping.[10] The improvements in cardiac perfusion and function were determined by cardiac magnetic resonance imaging.

Injections of bone marrow progenitor cells enriched with endothelial progenitor cells have also been given, with the aim of inducing increased angiogenesis and vasculogenesis in the infarcted tissue, to improve cardiac function and reduce apoptosis and ventricular remodeling.[7,9] These 2 studies, however, differed in treatment indication and in type of implanted cells. In the German study,[7] 20 patients with infarction in the acute phase undergoing reperfusion received intracoronary infusion of a heterogeneous population of cells, including a high percentage of peripheral blood endothelial or bone marrow progenitor cells cultured for four days *in vitro*. Patients who received cell therapy showed improved regional contractility as measured by echocardiography. Increased viability of the infarcted region was also demonstrated by positron emission tomography.[7] Stamm et al have recently reported the use of AC133 cells purified from the bone marrow of patients with an old infarction. A group of six patients underwent aortocoronary bypass surgery during which they received 1.5×106 AC133 cells directly into the peri-infarcted myocardium.[9] As with the other studies, there was an increase in cardiac contractility and perfusion of the infarcted area, as measured by radioisotope studies.

CONCLUSIONS

Despite the numerous experimental and clinical studies published to date, it still remains difficult to draw definitive conclusions. The interest in the use of cell therapy for heart failure is a result of expectations generated by this type of treatment. Nevertheless, the complexities of cell biology make great caution necessary when interpreting the results of this type of therapy, prior to clinical application. An additional aspect, which for reasons of space we are unable to discuss further, is the role of imaging techniques in determining the therapeutic benefit of this type of treatment.[41] Technical advances play an important role in designing clinical and experimental studies.

Perhaps the most important message here is the need for continued research in this field, and the need to facilitate multidisciplinary collaboration to enable some of the remaining questions to be answered. For example, what is the ideal source of stem cells? Do the different types of cell produce benefits in different situations? How many cells is it necessary to implant? What is the best route for stem cell administration —percutaneous, direct intracardiac injection, or intravenous? What are the indications for cell therapy in heart disease, i.e., is it better to administer the cells during the acute phase or in old infarctions? Can this therapy be used in the treatment of dilated cardiomyopathy? As we can see, there are far more questions than answers. We are facing a stimulating panorama in a completely new field which will doubtless lead to solutions and contribute to decisive improvements in therapy.

REFERENCES

1. Lagasse E, Shizuru, JA, Uchida N, Tsukamoto A, Weissman IL. Toward regenerative medicine. Immunity 2001;14:425-36.
2. Weissman IL. Stem cells: units of development, units of regeneration, and units in evolution. Cell 2000;100:157-68.
3. Weissman IL, Anderson DJ, Gage F. Stem and progenitor cells: origins, phenotypes, lineage commitments, and transdifferentiations. Annu Rev Cell Dev Biol 2001;17:387-403.
4. Poulsom R, Alison MR, Forbes SJ, Wright NA. Adult stem cell plasticity. J Pathol 2002;197:441-56.
5. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-González XR, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418:41-9.
6. Blau HM, Brazelton TR, Weimann JM. The evolving concept of a stem cell: entity or function? Cell 2001;105:829-41.
7. Assmus B, Schachinger V, Teupe C, Britten M, Lehmann R, Dobert N, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation 2002;106:3009-17.
8. Strauer BE, Brehm M, Zeus T, Kostering M, Hernández A, Sorg RV, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 2002;106:1913-8.
9. Stamm C, Westphal B, Kleine HD, Petzsch M, Kittner C, Klinge H, et al. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. Lancet 2003;361:45-6.
10. 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.
11. Menasche P, Hagege AA, Vilquin JT, Desnos M, Abergel E, Pouzet B, et al. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. J Am Coll Cardiol 2003;41:1078-83.
12. Kehat I, Kenyagin-Karsenti D, Snir M, Segev H, Amit M, Gepstein A, et al. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. J Clin Invest 2001;108:407-14.
13. Gepstein L. Derivation and potential applications of human embryonic stem cells. Circ Res 2002;91:866-76.
14. Zhang YM, Hartzell C, Narlow M, Dudley SC Jr. Stem cell-derived cardiomyocytes demonstrate arrhythmic potential. Circulation 2002;106:1294-9.
15. Beltrami AP, Urbanek K, Kajstura J, Yan SM, Finato N, Bussani R, et al. Evidence that human cardiac myocytes divide after myocardial infarction. N Engl J Med 2001;344:1750-7.
16. Laflamme MA, Myerson D, Saffitz JE, Murry CE. Evidence for cardiomyocyte repopulation by extracardiac progenitors in transplanted human hearts. Circ Res 2002;90:634-40.
17. Deb A, Wang S, Skelding KA, Miller D, Simper D, Caplice NM. Bone marrow-derived cardiomyocytes are present in adult human heart: a study of gender-mismatched bone marrow transplantation patients. Circulation 2003;107:1247-9.
18. Rosenthal N, Tsao L. Helping the heart to heal with stem cells. Nat Med 2001;7:412-3.
19. Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, et al. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. Proc Natl Acad Sci USA 2001;98: 10344-9.
20. Tomita S, Li RK, Weisel RD, Mickle DA, Kim EJ, Sakai T, et al. Autologous transplantation of bone marrow cells improves damaged heart function. Circulation 1999;100:II247-56.
21. Kamihata H, Matsubara H, Nishiue T, Fujiyama S, Tsutsumi Y, Ozono R, et al. Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. Circulation 2001;104:1046-52.
22. Kawamoto A, Tkebuchava T, Yamaguchi J, Nishimura H, Yoon YS, Milliken C, et al. Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. Circulation 2003;107:461-8.
23. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, et al. Bone marrow cells regenerate infarcted myocardium. Nature 2001;410:701-5.
24. Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J, et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nat Med 2001;7:430-6.
25. Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, et al. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. Nat Med 1999;5:434-8.
26. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275:964-7.
27. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143-7.
28. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. Circulation 2002;105:93-8.
29. Shake JG, Gruber PJ, Baumgartner WA, Senechal G, Meyers J, Redmond JM, et al. Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects. Ann Thorac Surg 2002;73:1919-25.
30. Reyes M, Lund T, Lenvik T, Aguiar D, Koodie L, Verfaillie CM. Purification and ex vivo expansion of postnatal human marrow mesodermal progenitor cells. Blood 2001;98:2615-25.
31. Seale P, Asakura A, Rudnicki MA. The potential of muscle stem cells. Dev Cell 2001;1:333-42.
32. Kessler PD, Byrne BJ. Myoblast cell grafting into heart muscle: cellular biology and potential applications. Annu Rev Physiol 1999;61:219-42.
33. Taylor DA, Atkins BZ, Hungspreugs P, Jones TR, Reedy MC, Hutcheson KA, et al. Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. Nat Med 1998;4:929-33.
34. Suzuki K, Brand NJ, Smolenski RT, Jayakumar J, Murtuza B, Yacoub MH. Development of a novel method for cell transplantation through the coronary artery. Circulation 2000;102:III359-64.

35. Robinson SW, Cho PW, Levitsky HI, Olson JL, Hruban RH, Acker MA, et al. Arterial delivery of genetically labelled skeletal myoblasts to the murine heart: long-term survival and phenotypic modification of implanted myoblasts. Cell Transplant 1996;5:77-91.
36. Chiu RC, Zibaitis A, Kao RL. Cellular cardiomyoplasty: myocardial regeneration with satellite cell implantation. Ann Thorac Surg 1995;60:12-8.
37. 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.
38. Perin EC, Dohmann HF, Borojevic R, Silva SA, Sousa AL, Mesquita CT, et al. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. Circulation 2003;107:2294-302.
39. Pagani FD, DerSimonian H, Zawadzka A, Wetzel K, Edge ASB, Jacoby DB, et al. Autologous skeletal myoblasts transplanted to ischemia-damaged myocardium in humans. J Am Coll Cardiol 2003;41:879-88.
40. Menasche P, Hagege AA, Scorsin M, Pouzet B, Desnos M, Duboc D, et al. Myoblast transplantation for heart failure. Lancet 2001;357:279-80.
41. Orlic D, Hill JM, Arai AE. Stem cells for myocardial regeneration. Circ Res 2002;91:1092-102.