Regeneration of the Myocardium
A New Role in the Treatment of Ischemic Heart Disease?

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Abstract—Intramyocardial cell grafting aims to limit the consequences of the loss of contractile function of a damaged left ventricle. Its functional efficacy is suggested by a wealth of experimental data using multiple evaluation techniques in different animal species. Intramyocardial injections of cultured fetal cardiomyocytes after infarction increase the ejection fraction. Cultured autologous skeletal myoblasts, which do not raise immunologic, ethical, tumorigenesis, or donor availability problems, improve ventricular function to a similar extent. The presence of connexin-43 is demonstrated between fetal (but not myoblast) grafted cells and host myocytes. Thus, the mechanisms of this beneficial effect (direct systolic effect, paracrine factors, passive girdling effect, and decrease in wall stress) remain controversial. These encouraging results have opened the way to the first clinical trial in patients with low ejection fractions, akinetic and nonviable postinfarction scars, and indications for coronary artery bypasses in remote, viable, and ischemic areas. Large-scale cell expansion allows a yield of $>10^9$ myoblasts from a single human muscular biopsy. Cultured autologous myoblasts are directly administered by multiple injections within and around the infarcted area during open-chest surgery. Preliminary postoperative observations show an improvement in ejection fraction, reappearance of a systolic thickening of the grafted scars, and a new-onset metabolic viability within this area. Thus, this new procedure might become a useful adjunct to current treatments of severe ischemic heart failure. (Hypertension. 2001;38:1413-1415.)

Key Words: myocytes ■ transplantation ■ myocardial infarction ■ heart failure ■ myocardium

Despite recent therapeutic advances, the prevalence of severe heart failure remains markedly high, estimated as 225 patients per million, with a rate of death of 35% per year. This has encouraged the development of alternative methods to medical therapies based on the regeneration of the pool of myocardial contractile cells. The transformation of nonmyogenic cells into contractile cells and the attempts to induce the cardiomyocytes to reenter the cellular cycle are, for the moment, out of reach. Myogenic cell grafting within the myocardium to limit the consequences of the loss of contractile function has emerged as a promising technique for severe postinfarction systolic left ventricular dysfunction. The first clinical application, which used cultured autologous skeletal myoblasts, has opened the way to randomized clinical trials.

History of Heart Tissue Regeneration

Transplantation of bone marrow cells has been successfully performed for decades in the treatment of hemopathies; the transplantation of skeletal muscle cells (Duchenne dystrophy), hepatocytes (as a bridge to liver transplantation), Langerhans islets (diabetes), or brain tissue (Parkinson and Huntington diseases) has also been attempted with inconsistent success. Like brain cells, adult ventricular myocytes are terminally differentiated, with no possibility of cell division; postinfarction ventricular remodeling becomes deleterious over time and does not resolve the problem of the loss of contractile cells.

If the reconstitution of the contractile cell pool seems conceptually attractive, there are potential limitations to cell transplantation within an infarcted myocardium. Because of thinning and fibrosis of the infarcted ventricular wall, catheter-based intramyocardial injections, as attempted with vascular endothelial growth factor, involve a risk of perforation or systemic emboli and have not yet been studied in this setting. Coronary administration has been proposed, but there is a potential for emboli. Thus, for the moment, the method of choice consists of cell injections during open-chest surgery with extracorporeal circulation.

A large number of potentially contractile cells are needed because an important part of the injected cells is rapidly destroyed (by immune reaction, apoptosis, and inflammatory process). Thus, a large-scale cell expansion is crucial before the procedure; ideally, the cells must possess a high potential for division in culture and for further multiplication after grafting. Immortalized cultured myogenic cell lines have been used experimentally but have a potential for tumor formation, which limits a potential human use. Fetal cardio-
Cardiomyocytes can be easily expanded in culture, but in a clinical perspective, they raise immunologic, ethical, and donor availability problems. Autologous myogenic cells are an optimal support because they obviate the need for immunosuppressive treatment. Skeletal myoblasts (which are also called satellite cells and are functionally indistinguishable from embryonic myoblasts) are stem cells located at the basal lamina of the adult skeletal muscle. They are highly resistant to ischemia and multiply after injury (each myoblast can divide by mitosis). An alternative option could be the use of cultured smooth muscle cells, which proliferate and undergo hypertrophy in situ. Human bone marrow cells might also be able to differentiate in vitro into cardiomyocytes and to regenerate infarcted myocardium in vivo, but their clinical use remains hypothetical, in part because of the difficulty of large-scale expansion.

Histological Studies
In vitro studies in animals have shown that transplanted cells within a normal or infarcted myocardium remain viable for months and can differentiate in situ; after engraftment, myoblasts merge into myotubes within the scar, with a decrease in fibrosis (Figure). In the case of grafted fetal cardiomyocytes, cells tend to align parallel to the host cardiomyocytes; connexin-43, desmoplakin, and cadherin, which are major adhesion and gap junction proteins of the intercalated disk required for cell-to-cell electrical coupling, are localized between grafted cells and between grafted and host cardiomyocytes. Thus, fetal cardiomyocyte grafts might contract in synchrony with the host tissue. In the case of skeletal myoblast grafts, cells can establish new muscle tissue (myotubes) when they are grafted into injured hearts. Moreover, this graft contracts when it is stimulated electrically, and because of its conversion to fatigue-resistant slow-twitch fibers, this new muscle may be suited for a cardiac-type workload. However, if undifferentiated rat skeletal myoblasts express N-cadherin and connexin-43, both proteins are markedly downregulated after differentiation into myotubes. Similarly, differentiated skeletal muscle grafts in injured hearts have no detectable N-cadherin or connexin-43. This beneficial effect has been observed to a same extent in fetal cardiomyocytes, allogenic fetal skeletal myoblasts, and autologous myoblasts; it is more marked in the case of lowest ejection fractions and is correlated with the quantity of injected cells. This effect has been observed in different species by the use of different evaluation techniques. As previously stated, it is not certain that improvement in left ventricular performance is mediated by an increase in systolic function due to synchronous contraction of the graft. An indirect systolic effect (mediated by angiogenic or growth factors secreted by the transplanted cells) is suggested by the improvement of global function of locally injected cells. A passive girdling effect, simply limiting progressive cavity dilatation and the decrease in pump function, is likely. Increased myocardial thickness due to local injections may decrease wall stress, thus improving function and reducing infarct expansion. However, recent experimental studies actually suggest a direct systolic effect of cell transplantation, with a regional reappearance of a systolic thickening of the infarcted (and injected) area by use of either echocardiography or sonomicrometry. Such a functional coupling does not, ipso facto, require connections between cells, because a simple stretch may initiate contraction. This is supported by observations that fibroblast transplantation improves only diastolic function, whereas myoblast transplantation improves diastolic and systolic performance.

Functional Studies
Regardless of the mechanism of its efficacy, the beneficial influence of the procedure on left ventricular performance has been suggested after cryoinfarction with the use of a Langendorff model in rats. Compared with control hearts, transplanted hearts showed no infarct expansion, the presence of new tissue (which occupied more than one third of the scar), and a higher developed systolic pressure. Multiple in vivo animal studies have demonstrated an increase in ejection fraction after intramyocardial injections of cultured myogenic cells in an infarcted area with, inconsistently, a decrease in cavity dilatation. This beneficial effect has been observed to a same extent in fetal cardiomyocytes, allogenic fetal skeletal myoblasts, and autologous myoblasts; it is more marked in the case of lowest ejection fractions and is correlated with the quantity of injected cells. This effect has been observed in different species by the use of different evaluation techniques. As previously stated, it is not certain that improvement in left ventricular performance is mediated by an increase in systolic function due to synchronous contraction of the graft. An indirect systolic effect (mediated by angiogenic or growth factors secreted by the transplanted cells) is suggested by the improvement of global function of locally injected cells. A passive girdling effect, simply limiting progressive cavity dilatation and the decrease in pump function, is likely. Increased myocardial thickness due to local injections may decrease wall stress, thus improving function and reducing infarct expansion. However, recent experimental studies actually suggest a direct systolic effect of cell transplantation, with a regional reappearance of a systolic thickening of the infarcted (and injected) area by use of either echocardiography or sonomicrometry. Such a functional coupling does not, ipso facto, require connections between cells, because a simple stretch may initiate contraction. This is supported by observations that fibroblast transplantation improves only diastolic function, whereas myoblast transplantation improves diastolic and systolic performance. Adult bone marrow mesenchymal stem cells, which communicate with the normal cardiomyocytes, beat synchronously in vitro but not in vivo, but they express...
cardiomyogenic phenotypes in vivo in rats after autologous implantation.26 Cardiomyocytes could also be derived from human embryonic stem cells, which can spontaneously contract in culture.27 Finally, adult bone marrow progenitor cells can induce vasculogenesis in the infarct bed and the proliferation of preexisting vasculature (angiogenesis), along with a parallel hemodynamic improvement.28

Preliminary Clinical Experience

These encouraging results have opened the way to the first clinical trial, started in France during the year 2000, by use of cultured autologous skeletal myoblasts, which do not raise immunologic, ethical, tumorigenesis, or donor availability problems.3 Inclusions concern patients with a low ejection fraction (<35%), akinetic and nonviable postinfarction scars (as assessed by dobutamine echocardiography and fluorescence-oxymyoglobin positron emission tomography), and indication for coronary artery bypass in remote, viable, and ischemic areas. Techniques have been developed that allow a yield of >10⁶ cells (of which >90% are myoblasts) within 2 to 3 weeks from a single human muscular thigh biopsy (vastus lateralis) weighing a few grams. Cells (suspended in a 5- to 8-ml volume) are directly injected in multiple sites (>30) within and at the borders of the scar area during open-chest surgery. Preliminary postoperative long-term (1 year for the first patient) follow-up shows an improvement in symptoms and an increase in ejection fraction; these observations could only be due to the confounding effect of the associated coronary surgery. However, appearance of a new contraction of the grafted and previously akinetic scar with a new-onset metabolic viability within this area (reflected by an increased uptake of fluoride-oxymyoglobin) suggests a beneficial effect of this new technique. Although these preliminary results should be interpreted cautiously, they support the clinical feasibility (from biopsy to resection after large-scale cell expansion) of autologous skeletal myoblast transplantation and raise the hope that this procedure might become a useful adjunct to current treatment for severe ischemic heart failure.

References


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