Cardiac reparation: fixing the heart with cells, new vessels and genes

P. Menasché and M. Desnos

1Department of Cardiovascular Surgery, Hôpital Bichat Claude Bernard, and 2Department of Cardiology, Hôpital Européen Georges Pompidou and Faculté de Médecine Necker-Enfants Malades, Paris, France

Cell-based interventions, angiogenesis and gene therapy are among the newest treatment modalities that have been proposed to improve outcomes in patients with ischaemic heart disease. Experimental data have established that implantation of contractile cells into fibrous post-infarction scars can allow those tissues to regain some functionality. Although clinical data are still preliminary, they support the concept of cell transplantation and raise realistic hopes that it will find a place among strategies to ameliorate heart failure in the future.

Therapeutic angiogenesis is another promising means of ameliorating ischaemic symptoms as there is already experimental evidence that angiogenic growth factors can stimulate the development of functionally significant new blood supply. In addition, successful correction of major abnormalities of calcium cycling by adenoviral gene transfer represents an encouraging finding in gene therapy. However, the complexity of gene dysregulation that is involved in heart failure complicates identification of the culprit genes, and important safety issues remain to be addressed before such therapies may proceed to clinical trials.

Key Words: Angiogenesis, cellular transplantation, gene therapy, heart failure.

Introduction

Cell-based interventions, angiogenesis and gene therapy are among the newest treatment modalities that have been proposed to improve outcomes in patients with ischaemic heart disease and, more specifically, those with cardiac failure. It is well documented that infarcted areas of the myocardium evolve toward fibrous scars that, if extensive, can lead to heart failure through excessive remodelling and reduced pump function. The clinical relevance of this problem is reflected in the high incidence of heart failure (approximately 120,000 new cases each year in France and 500,000 in the U.S.A.). The incidence of heart failure is expected to increase because of ageing of the population and improved survival after acute myocardial infarction.

This increase will add to the already enormous financial burden of heart failure, associated with the costs of lifetime treatment, nursing home care and repeated hospitalizations. Heart failure is already estimated to consume 1–2% of the total health care budget of Western countries\[^{1}\], and this figure is likely to continue to rise.

Improvements in medical therapy, primarily beta-blockers, angiotensin-converting enzyme inhibitors and aldosterone antagonists, have dramatically improved the prognosis of ischaemic heart failure. However, the overall outcome in heart failure patients remains dismal. A recent survey using the database of the U.K. National Health Service in Scotland\[^{2}\] indicated that, with the exception of lung cancer, heart failure has a worse 5-year survival than many common cancers. In the most severe, drug-refractory forms of cardiac failure, more aggressive approaches such as ventricular resynchronization and cardiac transplantation may be indicated.

The role of conservative surgical strategies (correction of mitral valve incompetence or restoration of left ventricular geometry) remains limited,
and implantation of left ventricular assist devices is still at a developmental stage. The limitations of all of these approaches mandate the search for alternate therapeutic options, such as cell-based interventions, angiogenesis and gene therapy, which are the focus of the present review.

Cell-based interventions

The objective of cellular therapy is to repopulate fibrous scars with new contractile cells, with the aim of restoring some functionality to these akinetic areas. This objective could theoretically be achieved through three distinct approaches: multiplication of residual myocytes, transforming fibroblasts in the scar, and implanting exogenous contractile cells.

Multiplication of residual myocytes

This approach would involve forcing residual cardiomyocytes to re-enter the mitotic cycle, thus expanding the number of contractile elements. Such a strategy has previously been considered unrealistic because adult cardiac cells were considered to be terminally differentiated and therefore unable to multiply. This assumption has now been challenged by experimental and clinicopathological studies that suggest that cardiomyocytes in infarcted or failing human hearts do retain the capacity to re-enter the cell cycle. However, the number of 'new' cells that can be generated appears far too low to compensate for the loss of cardiomyocytes that results from an infarct large enough to cause heart failure. An alternative approach involving residual myocytes might be to stimulate cardiomyocyte DNA replication by expression of transgenes that encode viral oncoproteins or endogenous cellular proteins that are involved in cell cycle control. However, such a strategy raises major safety issues that cast doubt on its clinical applicability.

Transforming fibroblasts in the scar

This approach would involve transforming the fibroblasts that constitute the post-infarction scar into contractile cells. Theoretically, this could be achieved by transfection with the MyoD master gene, which controls the skeletal muscle differentiation programme. Although there have been some successful experimental results, this approach is fraught with technical problems that render its clinical application unlikely in the foreseeable future.

Implanting exogenous contractile cells

To date, the most promising approach consists of implanting the scar with exogenous contractile cells. This strategy has been extensively investigated in the laboratory and is now being tested in the first clinical trials in humans. It should be noted that most experiments to date have focused on ischaemic, segmental cardiomyopathies (as does the present review). However, preliminary studies suggest that the putative benefits of cellular transplantation might extend to globally dilated cardiomyopathies, whether idiopathic or following doxorubicin therapy.

Myocyte transplantation: proof of concept

Transplantation of contractile cells (i.e. foetal cardiomyocytes or skeletal myoblasts) has been tested in small and large animal models of myocardial infarction generated by coronary artery ligation, endovascular coronary artery embolization, or cryoinjury. In those animal models myocyte transplantation has been shown to result in successful cell engraftment and improved function.

Evidence for cell engraftment has been obtained with various techniques, depending on the type of implanted cells. Foetal cardiomyocytes may be identified by pre-transplantation cell transfection with genes encoding beta-galactosidase (which gives cells a blue colour after appropriate histological processing). Alternatively, explanted specimens can be subjected to immunohistochemical analysis for the alpha-actin smooth muscle isoform, which is normally present in foetal but not in adult cardiomyocytes. A further approach (used in our own studies) is to use the Y-chromosome as a genetic marker for male foetal cells implanted into female myocardium.

Skeletal myoblasts are easier to identify than are foetal cardiomyocytes because they differentiate into typical multinucleated myotubes, which can be identified histologically. In addition, skeletal myoblasts can also be identified by pre-implantation labelling with a fluorescent dye (e.g. 4′,6-diamidino-2-phenylindole, which binds to the nucleus), or by staining with antibodies that are specific for skeletal muscle myosin.

Interestingly, engrafted myotubes display a composite phenotypic pattern, co-expressing embryonic, fast and slow myosin isoforms. By analogy to the events that occur following dynamic cardiomyoplasty, it may be speculated that stretching and/or repeated electro-mechanical stimulation triggers 'reprogramming' of engrafted myoblasts toward the expression of the slow fibre phenotype. A study reported by Chiu et al. supports the concept of 'milieu-induced differentiation'. However, our own studies of skeletal myoblasts do not support this paradigm, in that intramyocardially implanted myoblasts do not turn into cardiomyocytes (as demonstrated by their failure to stain positively for the cardiac-specific alpha-myosin heavy chain), but remain committed to a skeletal muscle phenotype.

Interestingly, transplanted foetal cells establish gap junctions with neighbouring host cardiomyocytes. This is not the case with skeletal myoblasts, however. Once skeletal myoblasts have differentiated into myotubes, they downregulate N-cadherin and connexin-43 (the major junctional protein of cardiomyocytes, which has been shown to mediate gap junction communication). Interestingly, it has been reported that human foetal cardiomyocytes express connexin-43, but not N-cadherin, whereas adult cardiomyocytes express both proteins. This suggests that engrafted foetal cardiomyocytes might be able to communicate with host myocytes via gap junctions.
As mentioned above, implanted cells have to possess contractile properties if they are to enable an improvement in function. Fibroblasts, for example, improve post-infarct diastolic performance, but are unable to augment contractile function\cite{15,16}.

**Foetal cardiomyocytes**

Foetal cardiomyocytes were the first cell type to be investigated for transplantation, and their successful experimental use has been pivotal to a convincing ‘proof of concept’. However, their clinical usefulness is undermined by problems related to ethics, availability and immunogenicity. The encouraging results obtained with intra-cerebral transplantation of brain tissue in patients with Parkinson’s disease cannot readily be extrapolated to the heart, which tolerates allografts much less well than does the brain.

**Skeletal myoblasts**

The limitations of foetal cardiomyocytes account for the current interest in skeletal myoblasts (satellite cells). These myogenic stem cells normally lie in a quiescent state under the basal membrane of skeletal muscular fibres. In case of injury, they are rapidly mobilized, proliferate and fuse to regenerate the damaged fibres. From the clinical point of view, skeletal myoblasts offer several advantages. First, their autologous origin enables large-scale clinical applicability. Second, it is possible to grow a large number of cells from a small biopsy. Third, they have a well-differentiated myogenic lineage, which minimizes the risk of tumourigenicity. Finally, they have a high resistance to ischaemia, which should enhance their survival after implantation into the hostile environment of the post-infarction scar.

Our findings show that, in spite of the lack of gap junctions with host cardiomyocytes, skeletal myoblasts improve function to a similar extent to that with foetal cells\cite{28}, justifying our choice of skeletal myoblasts for clinical trials. However, because engrafted myoblasts remain committed to their myogenic programming, it is conceivable that their contractile performance might be improved by pre-implantation engineering with genes encoding critical cardiac-specific proteins. Possible candidates include connexin-43 (which is a major constituent of gap junctions\cite{29}) and cardiac-type dihydropyridine membrane receptors (which are required for triggering a calcium-induced calcium release pattern of excitation–contraction coupling\cite{30}).

**Which cell type?**

As mentioned above, implanted cells have to possess contractile properties if they are to enable an improvement in function. Fibroblasts, for example, improve post-infarct diastolic performance, but are unable to augment contractile function\cite{15,16}. **Stem cells**

Myoblasts are not the only option for cell transplantation. Stem cells are currently the focus of growing interest (even...
Bone marrow stem cells
Bone marrow stem cells have 'pluripotentiality', which should allow them to differentiate into various cell types, including cardiomyocytes. In a clinical situation, these cells would have the advantages of autologous origin and easy retrieval via bone marrow aspiration (or even simple blood collection after pharmacological stimulation of the bone marrow).

It is unclear whether, before intra-myocardial implantation, bone marrow stem cells should first be cultured under conditions that promote differentiation into ‘cardiac’ cells or whether they should be transplanted unmodified, relying on local signals to drive them toward the cardiac phenotype. All experiments based on the former approach have used the compound 5-azacytidine. However, this ‘de-represses’ a wide array of genes, raising important safety issues should a human heart be injected with cells modified in this manner.

A second major question is whether bone marrow stem cells should be implanted regardless of type, or whether a particular subpopulation should be selected. At first sight, it seems attractive to use the CD34+ progenitors. However, because these only represent a small percentage of the total bone marrow cell population, the restricted number of cells available might not be enough for any significant improvement in cardiac function. It might be possible to expand them in vitro but this, in turn, might compromise their pluripotentiality. Pretransplantation mobilization of progenitor cells from the bone marrow by cytokines would then represent a more clinically relevant alternative. Another subpopulation of interest is the bone marrow stromal (mesenchymal) cells. Once implanted into a myocardial environment, these cells have been reported to receive signals that drive them toward cardiomyogenic differentiation[31]. However, the true ‘cardiac’ transformation of any type of bone marrow-derived cells must be carefully verified. If they only become ‘muscle-type’ cells, then native myoblasts would offer an easier option.

Evidence for cardiac differentiation has been provided by a recent study conducted by Orlic et al.[32]. Injection of a selected subpopulation of bone marrow cells (Lin- c-kit+POS) into an infarcted mouse myocardium resulted in their transformation into cardiac, smooth muscle and endothelial cells, and was associated with an improvement in function. This finding is theoretically important, but should be interpreted cautiously for two reasons. First, the small number of progenitors made it necessary to use cells from several mice to inject a single individual, thereby raising all the issues associated with allografting. Second, injections were made in a fresh infarct (3–5 h after coronary artery ligation). The microenvironmental signals that drove the cells toward the various reported lineages might be different from those that are present in the clinically relevant setting of an old post-infarction scar, as encountered in patients with heart failure.

Finally, it is interesting to note that, in the hamster model of dilated cardiomyopathy, cryopreserved bone marrow cells have been reported to be functionally less effective than similarly cryopreserved skeletal myoblasts[9].

Embryonic stem cells
The challenges posed by embryonic stem cells are even greater than with bone marrow stem cells. Aside from the ethical and regulatory problems, therapeutic cloning of mammalian cells is still fraught with major technical difficulties. The supply of human eggs is limited, and it is difficult for cloned eggs to reach the blastocyst stage. These problems make it unlikely that these ‘personalized’ cell lineages will be available in the near future. One alternative to the use of embryonic stem cells is to ‘reprogramme’ the patient’s own cells, rather than cloning them. They could be ‘rewound’ back to an embryonic stem cell-like phenotype, which could then be orientated toward the desired cell lineage. Another option might be to genetically engineer allogenic embryonic stem cells to make them match the intended graft recipient (and thus overcome an immune response from the host). These approaches are still at a very early experimental stage, however, and it is not possible to predict whether they will ever become clinically useful.

Stem cells versus myoblasts
This discussion of the problems associated with stem cell transplantation does not imply that unmodified, native skeletal myoblasts are necessarily the most appropriate choice for cell transplantation; they are simply the most practical choice at the moment. It is quite likely that skeletal myoblasts represent just one early step on the long journey of cellular therapy for heart failure. However, it appears that early optimism regarding stem cell transplantation is premature, because so many issues remain unresolved. Although an attractive concept, stem cell transplantation may take a considerable time to become a clinical reality. In contrast, skeletal muscle cell transplantation has already reached the stage of clinical trials.

Where should cells be injected?
Thus far, most experimental studies of any kind of cell transplantation in cardiac failure have used cell injection through multiple epicardial puncture sites. For example, this direct vision approach has been adopted in early clinical trials of skeletal myoblast transplantation (see below). However, the attractive possibility of delivering cells percutaneously through an endoventricular catheter is generating increasing interest among interventional cardiologists. This mode of delivery is made possible by recent improvements in catheter design and navigation systems. Its technical feasibility has now been established in animals and in humans. However, no data are yet available for cell survival following passage through these catheters, or for the functional efficacy of this ‘blind’ approach. In addition, the potential problem of cells being ‘squeezed’ by the heartbeat into the left ventricular cavity and subsequently migrating into the systemic circulation has not been thoroughly addressed.
The epicardial and endocardial routes are not mutually exclusive. A further mode of myoblast administration is via the intra-coronary route. This has been used successfully in situ in mice and in explanted rat hearts, but its clinical practicality remains debatable.

Regardless of the route chosen in future, it should be recognized that the physical process of injection still leads to a high cell death rate. Optimization of the procedure of cell transplantation will require improved delivery devices that enhance early post-injection cell survival. Alternatively, cell viability might be enhanced by blockade of apoptosis, promotion of angiogenesis, inhibition of matrix proteases, or even pre-implantation heat-shock treatment. Finally, major advances in tissue engineering also raise the possibility that cell engr~iftment could be achieved by seeding donor cells on biodegradable scaffolds. One of the most attractive applications of these ‘cellularized’ grafts is the repair of congenital heart defects.

How does cellular transplantation improve cardiac function?

The mechanisms by which cellular transplantation improves heart function still remain largely unknown. At least three hypotheses exist, which are not mutually exclusive, and are currently topics of intensive research.

Limitation of infarct expansion

It is conceivable that, through their elastic properties, implanted cells provide a ‘scaffold’ that limits post-infarct expansion, thus preserving the left ventricle from excessive remodelling. This hypothesis is strongly supported by the finding of reduced end-diastolic volumes in cell-transplanted hearts.

Intrinsic contractile properties

The observation that cell contractility is required for maximal functional benefit suggests that the intrinsic contractile properties of implanted cells are also important. In the case of foetal cardiomyocytes, the presence of gap junctions increases the likelihood of synchronous propagation of electrical impulses between host and grafted cardiac cells. The mechanism is more difficult to understand in the case of skeletal myoblasts, which lack these junctions. Conceivably, myoblasts could contract in response to the mechanical stress exerted by the surrounding cardiomyocytes, although this implies that both cell types are connected to the extracellular matrix through which the mechanical impulses would propagate. Myoblast ‘tethering’ to this matrix remains to be confirmed.

Release of growth and/or angiogenic factors

It is possible that transplanted cells might release growth and/or angiogenic factors that could enhance graft survival and stimulate contractile function in hibernating cardiac cells. This hypothesis is speculative and is not supported by experimental findings. Our studies show that myoblast transplantation fails to increase angiogenesis beyond that observed in control individuals receiving an equivalent volume of cell-free culture medium alone, through similar epicardial punctures. (Epicardial puncture alone can trigger angiogenesis, but the response is too small to be functionally relevant.) Attempts have been made to genetically engineer myoblasts to express the vascular endothelial growth factor (VEGF). This has been reported to result in increased angiogenesis and better haemodynamics as compared with unmodified myoblasts. However, another study found that injection of VEGF-transfected myoblasts into immunodeficient mice results in the formation of vascular tumours, raising doubts regarding the clinical applicability of this technique.

An early clinical trial of myoblast transplantation

The weight of experimental data on cellular and, more specifically, myoblast transplantation accumulated over the past decade led us to initiate the first phase I human trial. This was approved by the French Regulatory Health Authorities and our Institutional Ethics Committee in the Spring of 2000. The first patient received intra-myocardial injections of his own cultured skeletal myoblasts on 15 June 2000. The trial is completed and full results will be published separately. However, it is possible to make some general comments.

The primary objectives of the trial were to assess the feasibility and safety of the procedure. Efficacy was only a secondary end-point, given the lack of a control group and the potentially confounding effect of the concomitant bypass. Therefore, the study does not allow us to draw any definite conclusions regarding the specific effects of myoblast transplantation on functional outcome. However, functional outcome will be a primary end-point of a multicentre prospective placebo-controlled randomized phase II trial planned for 2002.

Eligibility for inclusion in the phase I trial was based on the following: impairment of left ventricular function, as assessed by dobutamine echocardiography and fluoro-deoxyglucose positron emission tomography; and an indication for concomitant coronary artery bypass grafting in remote (i.e. different from the transplanted area) and viable, but ischaemic myocardium.

The trial followed a straightforward three-stage protocol. First, a muscle biopsy was taken from the thigh under local anaesthesia. Second, the minced muscle was grown for
2–3 weeks in the cell culture laboratory using customized techniques in order to obtain a pure (at least 50% myoblasts) and high (at least 400 x 10⁶) cell yield. Microbiological controls were performed throughout this expansion phase. Third, the cells were reimplanted into the post-infarct scar, while the chest was open for coronary artery bypass grafting. On completion of the bypass graft, the cells (which had been concentrated into 4–6 ml fluid) were injected at 25–50 sites in the post-infarct scar. A pre-bent microneedle was used to create subepicardial ‘pockets’, thus avoiding inadvertent intra-cavitary cell delivery. The transepicardial injections were done according to a ‘virtual grid’, covering the entire area of scar tissue. It is evident that the procedure is feasible (assuming the availability of adequately equipped Good Manufacturing Practices facilities and expertise in large-scale cell expansion for clinical purposes). The key to technical success is the quality of cell cultures.

With regard to safety, bleeding from the multiple puncture sites does not appear to be a problem, either intra- or post-operatively. However, ventricular arrhythmias are a potential concern. Although they have not been observed in our animal experiments with skeletal myoblasts or with AT-1 cardiomyocytes derived from a differentiated tumour line [42], they have occurred in some of our patients. The mechanism of these arrhythmias (circus rhythm or ectopic pacemaker) has not yet been elucidated. Post-infarction scars in patients represent a potentially arrhythmogenic substrate, which can never exactly be duplicated in animal models. The potential risk for ventricular arrhythmias has led us to implement some safety measures. We now start amiodarone prophylaxis at the time of biopsy and continue until 3 months post-operatively. Patients also receive close in-hospital monitoring for at least 2 weeks (to cover the period during which most of these arrhythmias are likely to occur).

A further issue that is relevant to arrhythmias is the avoidance of intra-myocardial ‘over-cellularization’. Optimizing the number of cells to be delivered is difficult. On the one hand, it is known that up to 90% of injected cells die within a few hours after implantation. Hence, we have transplanted a large number of cells (up to one billion) in order to compensate for this high attrition rate and to maximize the chance of an improvement in function. On the other hand, increasing the number of cells increases the volume of injection and the number of puncture sites. This may amplify the inflammatory response to needle punctures and the subsequent clearance of dead cells. Compounds released by inflammatory cells that invade the transplantation areas (particularly cytokines and nitric oxide) could increase the vulnerability of the myocardium to arrhythmias. Future dose-ranging studies should help in ‘fine-tuning’ the number of cells required to obtain the optimal risk–benefit ratio. In the meantime, ongoing laboratory experiments should help in gaining an understanding of the mechanisms of arrhythmias and should assist in developing appropriate preventive strategies.

Any efficacy data obtained from this phase I study will be difficult to interpret because of the methodological limitations outlined above. A confounding effect of the concomitant revascularization can never be completely ruled out. However, preliminary findings do appear to show that scar tissue implanted with myoblasts can regain functionality, supporting the concept of cellular transplantation as a means of augmenting heart function.

**Angiogenesis**

Angiogenesis, which provides new blood supply to the diseased heart, is not designed primarily to improve the function of the failing myocardium, but to relieve ischaemic symptoms in patients who are unsuitable for more conventional forms of revascularization (angioplasty or bypass surgery). Proof of concept for angiogenesis has been obtained from animal models of myocardial ischaemia. Compelling data show that administration of angiogenic growth factors such as VEGF and basic fibroblast growth factor (either as recombinant proteins or by gene transfer) can increase myocardial blood supply through neovascularization.

The initial clinical trials with these factors [43] yielded mixed, although generally encouraging, results. A recent placebo-controlled study [44], in which naked plasmid DNA encoding VEGF-2 was injected through an endoventricular catheter, reported improved myocardial perfusion, paralleled by a reduction in angina in the treated group. However, several issues remain to be addressed, including the nature of compounds to be administered (gene or protein), the optimal dose schedule (single or repeated administration) and the route of delivery. Percutaneous catheter-based intra-coronary or endoventricular administration is one delivery option. Another is a direct surgical approach using direct intra-myocardial injections or subepicardial encapsulation of sustained-release polymers [45]. These various approaches must be evaluated in terms of maximizing drug distribution and retention in the target myocardial tissue. However, they must also be evaluated with reference to adverse events, in particular atherosclerotic plaque expansion/destabilization, development of functionally abnormal blood vessels, proliferative retinopathy, the risks associated with viral vectors, and (albeit less likely) acceleration of occult malignancies [46]. These issues were comprehensively reviewed by an Expert Panel Report of the Angiogenesis Foundation and the Angiogenesis Research Center [47].

At the basic science level it is interesting to note that, when naked plasmid DNA encoding the 165-amino-acid isoform of human VEGF was injected into the myocardium of patients with chronic myocardial ischaemia, there was a rise in plasma levels of VEGF. This resulted in mobilization of endothelial progenitor cells. These could home in foci of neovascularization and differentiate into endothelial cells [48]. This finding raises the hypothesis that the therapeutic development of new blood vessels may not be restricted to ‘classical’ angiogenesis (defined as the proliferation and migration of fully differentiated endothelial cells). It could also occur through enhanced vasculogenesis, which is the primary process responsible for the growth of vasculature in the embryo.
Gene therapy

Several experimental studies provide convincing evidence that gene therapy can be an effective means of improving the function of the failing heart. The complexity of the problem differs markedly, however, depending on the specific type of heart failure. Gene therapy may be predicted to be most successful in monogenic disorders such as familial dilated or hypertrophic cardiomyopathy. The validity of this approach is demonstrated by the efficacy of a recombinant adeno-associated virus-mediated transfection of the gene encoding delta-sarcoglycanin reversing morphological and functional alterations in the hamster model of diluted cardiomyopathy[49].

The situation is far more complex in the case of ischaemic heart failure, which results from the dysregulation of several signalling pathways, complicating the identification of candidate genes. Nevertheless, genetic manipulation of three major areas has been investigated in ischaemic heart failure[50]: calcium handling, beta-adrenergic signalling and apoptosis. Abnormalities of calcium homeostasis associated with heart failure were addressed by del Monte et al.[51]. In cardiomyocytes from failing human hearts, those investigators showed that adenoviral gene transfer resulted in over-expression of SERCA2a (the pump that contributes to calcium removal from the cytosol) by re-accumulating it in the sarcoplasmic reticulum. This led to an increase in both protein expression and pump activity, accompanied by normalization of the major abnormalities of calcium handling.

It remains uncertain whether such improvements in contractile parameters at the cellular level can affect left ventricular function and ultimately survival of patients with advanced heart failure. However, encouragingly, the same group[52] also found that over-expression of SERCA2a induced by gene transfer in vivo was effective in normalizing systolic and diastolic function in a rat model of pressure-overload hypertrophy in transition to failure. Other studies have shown that adenoviral gene transfer of beta-2-adrenoreceptors or an inhibitor of beta-adrenoreceptor kinase 1 restores beta-adrenergic signalling. However, the clinical utility of this approach might be hampered by possible adverse effects associated with a sustained increase in cytosolic cyclic adenosine monophosphate (cAMP)[53].

Apoptosis represents another potential target for gene therapy. Programmed cell death pathways can be blocked by adenoviral gene-transfer-induced over-expression of antiapoptotic agents such as Bcl-2, or compounds that potentiate the antiapoptotic effect of some growth factors (e.g. phosphatidylinositol-3-kinase and insulin-like growth factor-1). All of the strategies described thus far target genes that are functionally impaired or defective. However, inducing expression of genes in tissues where they are normally silent may offer an alternative approach. For example, a recent study[53] has reported the recruitment of cAMP-dependent contractility in response to transfection of the myocardium with the V2 vasopressin receptor genes (which are normally expressed only in the kidney).

In spite of all these encouraging results, there is still a wide gap between laboratory findings and the clinical application of gene therapy in heart failure. Expected improvements in vector technology and gene delivery systems will no doubt help to fill this gap. However, studies in vitro or in rodents still need to be extended to large animal models before proceeding toward clinical trials of gene therapy[54]. Although the message conveyed by a recent enthusiastic review[55] is that many of the concerns raised by cardiac gene therapy are unfounded, most clinicians and regulatory authorities would still consider that important safety and efficacy issues remain to be addressed. Such issues include control of the targeted protein expression as well as anticipation of potential adverse outcomes, particularly inflammation, autoimmunity and oncogenesis. Clarification of these questions is of crucial importance; in contrast to pharmacological interventions, which can be terminated if untoward effects become evident, gene therapy is basically irreversible once implemented. Whatever the eventual place of gene therapy in heart failure, however, studies in this area are already contributing to a better understanding of the pathophysiology of the disease and to the identification of new molecular targets for classical drug therapies.

Conclusion

In conclusion, skeletal myoblast transplantation is the cell-based intervention that is most likely to become applicable in clinical practice in the immediate future. As outlined in the report of the workshop on cellular transplantation held under the auspices of the U.S. National Heart, Lung and Blood Institute[56], several key questions still need to be addressed. These include the advantages and disadvantages of different donor cells; the extent to which cell engraftment affects cardiac function actively (by increasing contractility) or passively (by limiting infarct expansion and remodelling); the development of strategies to enhance cell survival; and the identification of cardiac diseases for which cell engraftment may be beneficial. The huge amount of research in this area should provide answers to these key questions in the near future.

As research progresses, it is likely that cellular therapy will also take advantage of advances in gene discovery and developmental biology. Genetic manipulation of donor cells to make them express cardioprotective recombinant proteins, or the delivery of genes that encode proteins that are involved in angiogenesis are just two examples of such an interplay. Thus, cellular transplantation, angiogenesis and gene therapy must not be viewed as separate entities. In fact, they are likely to be mutually beneficial and complementary. By taking advantage of all three approaches, we may develop combined strategies that will significantly improve outcomes in patients with heart failure.

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