Myoblast transplantation can repair heart damage

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Abstract Myocardial regeneration is an exciting new frontier for the treatment of heart disease. Many approaches are currently being tested. The use of autologous skeletal myoblasts has been the earliest, with over 10 years of research having been conducted. Current progress in the area of skeletal myoblasts for cardiac regeneration is presented. Reviewed is work from both pre-clinical and clinical studies. Work in this area continues to progress and definitive studies to assess efficacy of myoblasts for heart failure either have been initiated or will be initiated shortly. One result that is clear is that myoblasts can survive and form myotubes and myofibers in the area of myocardial infarction. In the early clinical trials, arrhythmia was a concern. However, further studies have shown that the risk was assumed prematurely based on limited human studies. Myoblasts, therefore, provide a highly promising treatment for heart disease. (J Geriatr Cardiol 2006;3:168-70.)

Key Words myoblasts transplantation; heart failure; skeletal myoblasts

Advantages of myoblasts

There are an array of different cell types today that have been used in animal models and in preliminary human clinical trials to regenerate cardiac tissue.\(^3\) Skeletal muscle myoblasts provide several advantages over other cell types. Skeletal muscle cells, unlike heart muscle, retain regenerative potential attributable to the presence of stem or satellite cells. Resident within adult skeletal muscle is a pool of undifferentiated mononuclear cells, myoblasts. Because of their anatomic location at the periphery of the mature, multi-nucleated myofiber, they have also been called satellite cells.

The effectiveness of any cell in effecting tissue repair will depend on a number of factors. Some of these factors include 1) the cells should be easily prepared under controlled, well defined procedures that guarantee consistent, viable cell isolates; 2) sufficient numbers of cells need to be delivered or make their way to the site of damage; 3) cell survival must be sufficient to repair a significant portion of the damaged heart to sustain long term rather than just transient recovery; 4) cells must be able to survive in myocardial scar with poor arterial blood supply; 5) cells must not be subject to immune mediated rejection and 6) cells must not adversely affect electrical conduction within the heart. Extensive experimentation, in multiple labs across the world has shown that autologous skeletal myoblasts exhibit all these properties. Myoblasts can be expanded in culture to yield billions of cells that maintain muscle properties consistently and repeatedly. Cell survival in sites of heart damage has been consistently achieved.\(^3\)

Myoblasts are ischemia resistant and adapted to survive in sites of tissue damage and inflammation. They also do not express tissue factor, which on other cell types can act to induce microembolization due to stimulation of clot formation. Finally, myoblasts form muscle regardless of the location that they are placed.

Animal studies

Animal studies with transplantation of skeletal myoblasts into the ischemically damaged heart have demonstrated that skeletal myoblasts engraft, contribute to improved function, and survive long-term, all without any adverse effects on cardiac function such as arrhythmia.\(^3\)

Further, survival of myoblasts within infarcted hearts has been examined thoroughly, both functionally and histologically. Studies have confirmed that autologous rat myoblasts form stable grafts in ischemically damaged myocardium, and enhance myocardial function as measured by a Langendorf procedure.\(^3\) The cells survived both outside and inside the infarct zone. In addition, the cells fused to form myotubes and appeared to form close contact with the myocytes at the borders of the infarct. Increased
myocardial contractility and cardiac output as compared to control animals was observed as well as prevention of increased ventricular volume or remodeling. Furthermore, myoblasts injected into healthy heart tissue engraft, form myotubes and produce no adverse effects. Finally, autologous myoblast transplants in ischeci-cal damaged sheep myocardium survived up to one year post-transplant, formed myotubes that stained for skeletal muscle markers and improved multiple cardiac functional parameters that remained improved until the end of the study at one year.

**Alternative cell sources**

Many, if not all, of the following alternate approaches have shown some promise in pre-clinical animal testing. However, the data for all these other cell types is more limited than that for myoblasts, and in cases where the effects of myoblasts and other cell types have been tested side by side, only fetal cardiomyocytes have performed better. Because cardiomyocytes cannot be grown in culture, there is no practical, readily available source. Investigation is currently underway to isolate and expand cardiomyoblasts from cardiac biopsy.

**Hematopoietic stem cells (HSC):** Continued conflicting data relative to survival and differentiation of HSC has been reported. More data substantiating the capabilities of HSC to differentiate and repair damaged myocardium need to be generated to satisfy the growing number of studies that have failed to confirm earlier studies. The stimulation of bone marrow and isolation of CD34 from peripheral blood is currently under Phase II Clinical trial sponsored by Baxter.

**Fetal cord blood cells:** Fetal cord blood cells are autologous cells, but these cells would have to be stored for several decades before use and there are limited data as to their effectiveness.

**Human bone marrow derived mesenchymal stem cells (MSCs):** The MSCs can be isolated from bone marrow. One can obtain almost one thousand treatments from a single donor. Although these cells are allogenic, early pre-clinical studies demonstrated that MSCs might modulate the immune system. MSCs were used in the Phase I Clinical trial on 50 patients without the use of immunosuppression. MSCs are used in patients who had recent heart attacks otherwise differentiation signals may result in exacerbation of scar formation. Finally, there is limited evidence for cell survival in pre-clinical animal models, and, thus, mechanism of action is still unclear.

**Human embryonic stem cells (hES):** In laboratory experiments, hES can form cardiac cells, but after transplant to the mouse heart tumors developed. Purification of cardiac cells free from undifferentiated hES must be 100%, otherwise unwanted cell types can develop within the grafts. Finally, allografts would be required and therefore immunosuppression would also be required.

**Combination approaches**

Although success in animal models has been obtained with various cell transplantation strategies, there have been similar successes with various gene therapy and growth factor therapy studies. Despite successes in animal studies, gene and growth factor approaches have so far proved ineffective in patient studies. Cell therapy may suffer the same fate in clinical studies; therefore, approaches that can combine the benefits of cell therapy with benefits from gene or growth factor therapy would be appropriate. Some studies of this kind have already been performed in animals and combined therapies have proven to be more effective than the individual component approaches. A comparison of the best combination therapies would aid in identifying the best strategy to move forward into patients.

Studies have demonstrated in an animal model that pre-treatment of myocardial scar with vascular endothelial growth factor (VEGF) 3 weeks prior to cell implantation significantly enhances scar vascularization and perfusion, and the survival and functional efficacy of implanted cells, as demonstrated by echocardiography and exercise testing. It has also been demonstrated that transfection of cell with a cell survival factor, such as Akt, or angiogenic factor such as VEGF can also enhance their survival, and thus a combined angiogenic (or arteriogenic) and anti-apoptotic therapy holds promise in this regard as well. Further, while the administration of growth factors has provided encouraging evidence of our ability to induce therapeutic neovascularization, much additional data suggest that EPC's may offer additional advantages in inducing new vessel growth. There are a multitude of potential combination therapies to be tested, but there are accepted animal models in which these approaches can be tested and compared. With careful animal testing, the most promising combinations can be brought forward into patients, thus limiting the number of potentially failed clinical trials and the loss of patient and physician enthusiasm to participate in trials of emerging repair strategies.

**Current myoblast clinical studies**

There are multiple ongoing or recently completed clinical studies utilizing autologous skeletal myoblasts (Table 1). Initial concerns were raised after only a small number of patients had received myoblasts that there was a risk of arrhythmia related to their use. Subsequent studies have not confirmed that hypothesis and myoblast treatment carries the same risk for arrhythmia as any other heart procedure in this patient group. The recent MAGIC trial in Europe was the largest study of its kind and no risk of increased arrhythmia was observed in this trial.

Unfortunately, the MAGIC trial was concluded early due to lack of evidence for efficacy. This most likely was related to trial design and not to lack of benefit from myoblasts themselves. The MAGIC trial had the difficult task of showing benefit of myoblasts over concomitant coronary artery bypass surgery. We describe here a different approach to a myoblast clinical study that utilizes intraventricular catheter injection of myoblasts. This is an extension of the largest series of clinical studies with myoblasts performed in the US.

Currently, a randomized study of 24 patients with congestive heart failure (New York Heart Association Classification II-IV) due to previous myocardial infarction is in progress at the Arizona Heart Institute. Patients are randomized 1:1 to
undergo percutaneous endomyocardial autologous myoblast transplantation (Treatment) and maximal medical therapy or continue on maximal medical therapy only (Control). The cells are injected percutaneously into the endoventricular surface of the previously infarcted left ventricle using 3-D mapping and injection system (Webster Biosense NOGA and Cordis Myostar injection catheter).

Treatment patients are monitored as in-patients during the transplantation procedure and for the first 24 hours thereafter. Vital signs and cardiovascular parameters are extensively monitored during the first 24 hours to determine the acute feasibility and safety of the transplantation procedure. The patients are then monitored over a period of 12 months to determine the long-term safety and efficacy of transplants.

To date, 19 of the planned 24 patients have been enrolled. The transplants have been well tolerated and the injection procedures have all been successful and without complications. We will continue to enroll and test more patients to determine if patient outcomes are consistent with complications. We plan to initiate a further double-blind placebo controlled study in the near future.

Table 1. Recently completed clinical studies utilizing autologous skeletal myoblasts

<table>
<thead>
<tr>
<th>Trial</th>
<th>Number of patients enrolled</th>
<th>Injection method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pagani 2003</td>
<td>6</td>
<td>Epicardial, Open Chest</td>
</tr>
<tr>
<td>Menasche 2003</td>
<td>10</td>
<td>Epicardial, Open Chest</td>
</tr>
<tr>
<td>Siminiak 2004</td>
<td>6</td>
<td>Epicardial, Open Chest</td>
</tr>
<tr>
<td>Siminiak 2005</td>
<td>10</td>
<td>Transvascular</td>
</tr>
<tr>
<td>Smits 2003</td>
<td>5</td>
<td>Endocardial</td>
</tr>
<tr>
<td>Ince 2004</td>
<td>12 (6 treatment, 6 control)</td>
<td>Endocardial</td>
</tr>
<tr>
<td>Dib 2005</td>
<td>24</td>
<td>Epicardial, Open Chest</td>
</tr>
<tr>
<td>MAGIC Trial 2006</td>
<td>300 planned (2:1 treatment to control), terminated after 100(+) enrolled</td>
<td>Epicardial, Open Chest</td>
</tr>
<tr>
<td>Herreros 2003</td>
<td>12</td>
<td>Epicardial, Open Chest</td>
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References