Reviews

Recent Advances in Cell and Gene Therapy of Ischemic Cardiomyopathy

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Key words: **Heart failure, cell therapy, cell transplantation.**

Manuscript received: May 27, 2002;
Accepted: March 4, 2003.

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Among the many causes of heart failure, the most common is coronary artery disease. Myocardial cells, as considered until now, can't multiply or regenerate[1]. As a result, the irreversible ischemia (infarction) results to progressive replacement of the dead myocardial cells by fibrous tissue (scar), to reduction of systolic and diastolic function of the heart and to initiation of the clinical syndrome of heart failure with prominent neurohormonic activation.

The pharmacological therapy of heart failure includes many drugs, such as angiotensin-converting inhibitors, angiotensin II-receptors antagonists, diuretics, β-adrenergic blockers and digitalis. Some of these agents offer only symptomatic relief, while others (as it has been proven by multicenter trials) improve survival. In spite all this, medical therapy can't revert the structural cause of heart failure, which is the permanent loss of myocardial tissue.

The surgical approach treatment of heart failure is also of limited value. The most effective surgical method of end-stage heart failure is heart transplantation but the serious lack of transplants minimizes the use of this method[2]. Coronary artery by-pass grafting may be of great value for some patients with ischemic cardiomyopathy. Nevertheless, this therapy is useful only for patients with viable myocardium and anatomically appropriate for revasculization vessels and so the majority of patients can't gain any benefit, since this therapy doesn't restore the structural anomalies of heart failure[3]. The various left ventricular assist-devices, can't stand as permanent solution and are mostly used as a bridge for transplantation, because of the infections, coagulation disorders and other serious complications[4].

New methods of heart failure treatment after myocardial infarction

The restrictions, that we have mentioned above, for the drastic treatment of heart failure due to myocardial necrosis after infarction, turned many researchers to cellular treatments, in order to improve myocardial contractility.

These efforts followed four main directions: (Table 1).

1. Stimulation of host cardiomyocytes that have survived in the infarct area, in order to start multiply and replace the fibrous tissue.
2. Conversion of the fibroblasts of the scar tissue into myocytes.
3. Cell transplantation.
4. Genetic modification of the failing cellular functions.

In this paper, we will review the current advances of this promising heart failure treatment.

Table 1. Methods of cellular treatment of heart failure mentioned in the text with their corresponding references.

Cellular treatment methods		References
1 Stimulation of host cardiomyocytes		1, 5
2 Conversion of the fibroblasts into myocytes		6, 7, 8
3 Cell transplantation	Satellite cells (myoblasts)	9, 10, 11, 12, 13, 14, 15, 16, 17
	Bone marrow cells.	18, 19, 20, 21, 22, 23, 24
	Smooth muscle cell	25
	Fetal cells	26, 27, 28, 29, 30, 31
	Cardiomyocytes (autologous transplantation)	32, 33
4 Genetic modification		34, 35, 36, 37

1. Stimulation of survived host cardiomyocytes

The aim of this method is to achieve the multiplication of the cardiomyocytes that have survived from a myocardial infarction and exist as islets into the scar tissue or surround the scar. It is until today believed that the human myocardial cells are getting during the birth out of the cellular cycle of reproduction and multiplication and stay in a hypnosis stage during life. So, practically there is no possibility of replacing the cells that are dying during a myocardial infarction. The heart development after birth is possible only through the hypertrophy of the myocardial cells. In spite all this, in a recent study of Beltrami et al[5] a degree of mitosis was found in myocardial cells, which was much more intensive in the cells that were close to the myocardial scar than those in areas away from the scar. Unfortunately, the degree of the myocardial cells regeneration is minimum compared to the infarction necrosis. Furthermore, It has been achieved stimulation in animal myocardial cells cultures. These cells passed to DNA transcription phase (phase S of the cellular cycle). Until now it has not been achieved similar progress in human myocardial cells, but it seems possible and the research will continue, without neglecting the risks of this effort (tumors development)[1].

2. Conversion of cardiac fibroblasts into myocytes.

A second approach for replacing the scar with functional myocardial cell is the conversion of the fibroblasts that lie in the fibrous tissue into myocytes.

The MyoD gene is expressed with the adjustment of the differentiation of the skeletal myocytes. The researchers basic assumption was that with the action of the MyoD gene the fibroblasts could be converted into myocytes.

During the first experiments[6] the MyoD was transported through a retrovirous carrier to fibroblasts from mice heart. A few days later the fibroblasts showed characteristics of skeletal myocytes. They were elongated, they obtained many nucleus and tubulus resembling to sarcoplasmic reticulum and they generally started to look like cells from striated muscles.

Later on, the virus carrier MyoD was infused into dogs' heart, in the site of a previous infarction. In a few days the existence of rapid myosin at the infusion area was detected. But this indication of skeletal cell differentiation was not accompanied by other histological indications.

In another study[7], the transport of the myogenin gene (of the MyoD family) through an adenovirus carrier to fibroblasts culture caused again a successful conversion into cells that look like skeletal muscle cells. Unfortunately, after infusion into mice heart (and destruction of the thymus gland) neither histological indications of fibroblasts conversion into myocytes were found, nor myosin was detected.

In a third study[8], there was a direct transport of the MyoD gene into mice heart. The infusion of the DNA of the MyoD resulted in the detection of heavy chains of foetal skeletal myosin in the area of infusion.

Thus, the studies until now have shown that is possible, in the infarct scar area, the conversion of the fibroblasts into cells with skeletal tissue characteristics. Nevertheless, no functional results of this conversion has been detected, especially taking into consideration the structural and the functional differences between skeletal myocytes and myocardial cells.

3. Cell transplantation

The third and rather most promising method is cell transplantation in the area of the myocardial scar. The cells that are used may come from different sources, but the use of cells from the patient itself is preferable because it saves us from life-long immunosupresion. A restricted factor for the autologus transplantation is the time needed for extraction, culture and implantation of the cells in order to have the desirable result. Other methods, such as cell transplantation from animals or donators, contain risk of life-long immunosupresion, infections etc.

a) Transplantation of satellite cells

As satellite cells or myoblasts we consider the stem cells existing in the skeletal muscles. In case of wounding or loss of muscular tissue they activate fast, they multiply and finally restore the damage.
In a primary study[9], the researchers cultured for 2-3 weeks satellite cells of dog muscular tissue. Myocardial infarction was caused to these dogs and then satellite cells were implanted in the scar of every donator dog. When the scar was reexamined after one and two months, the existence of intercalary disks and syncytium, which are characteristics of a normal myocardium, was detected in 2 of the 5 animals.
Other researchers[10], implanted cultured myoblasts taken from mice's skeletal muscles into their left ventricles. It was observed, in 4 of the 24 mice, a gradual differentiation of the myoblasts into muscular fibers, which were synchronized with the surrounded myocardial fibers.

In other study [11], the transplantation of skeletal myoblasts in mice's heart scar leaded in two weeks to the detection of myofibrils with sarcomeres. These muscular implants in samples, showed capability of contraction after stimulation with electricity, proof of contractile proteins existence.

The communication between the grafted skeletal myocytes and the native myocardial cell is probably the key for the acquisition of myocardial phenotypes from the skeletal cells. In a recent study[12], the researchers grafted skeletal myoblasts in a scar area of rabbits' heart. The different evolution of the grafted cells was discovered with immunochemical and histological tests. In the center of the scar, myoblasts were developed into skeletal myocytes. On the contrary, in the scar periphery (close to the healthy myocardial tissue), were detected immature myocardial cells. The cause and the mechanism of this phenotypic differentiation of the grafted cells hasn't been clarified yet.

In a recent research of Scorsin et al.[13] the effectiveness of myoblasts and embryo myocytes in mice's hearts after a myocardial infarction was compared. The improvement of left ventricular ejection fraction (echocardiographically assessed) in both groups was similar and it was significantly increased compared with the control group, (with no cell transplantation). This is one of the few comparative studies between two different methods of cellular treatment.
Menaschè et al.[14] recently announced their observations from implantation of myoblasts in an infarctional scar of a patient during coronary artery bypass. This was a patient with heart failure (ejection fraction <35%) and the aim of the study was to evaluate in clinical practice the technique of extraction, multiplication and transplantation of the myoblasts and not the effectiveness of the method, which will be the aim of a multicenter trial in the near future. In this particular patient, viable and functional muscular tissue at the scar zone was detected with the use of positron emission tomography (P.E.T) and echocardiography, respectively.

Dib et al[15], recently presented the results of myoblasts transplatation in myocardial scar, in patients with ischemic cardiomyopathy during a coronary artery by bass surgery, or during implantation of a left ventricular assist device. The study included 16 patients. Almost 2 grams of muscle tissue were taken from each patient's thigh. Myoblasts, after their separation, were multiplied in vitro and finally 10 to 300 million cells were injected in each patient. The researchers stated that during follow-up they detected that the transplanted cells remained alive and that significant improvement of ejection fraction was achieved.

The studies mentioned above, generally prove that myoblasts transplantation is one of the most hopeful techniques. This is an autologous transplantation and we have no rejection or immunosupresion problems. Their multiplication is easy and it is mentioned that they have a relatively increased resistance to ischemia. Finally, the risk of malignant tumors development is reduced, as a well-differentiated cellular group is involved.

The most important problem for the myoblasts transplantation seems to be the deficiency or the unsatisfactory development of the intercalary disks that connect the grafted tissue with the myocardium. This insufficient communication and the increased tension

to the differentiation to skeletal muscular tissue, are the obstacles for the myoblasts to become full functional for the heart. It is believed that the genetic modification of the myoblasts before their transplantation will may help overcome these two problems[16-17].

b) Transplantation of stem cells from the bone marrow

The extraction of stem cells from the bone marrow has the advantage that they are totally indifferentiated. There are three methods for transplanting these cells. The first one is by culture and process in a way that their differentiation to myocardial cells will be stimulated. Until today this is achieved with the use of 5-azacytidine. This method bears the risk of an uncontrolled activation of various gene groups and the risk of cancerogenesis, after the transplantation. The second method is the direct transplantation of these cells in the scar zone (after their in vitro multiplication) and the expectation of their differentiation by signals that they will receive from the surrounded myocardial tissue. The third method is the transplantation of selected cell groups, such as the $CD34^+$, that they have more possibilities of suitable differentiation, but there is the problem of their small concentration in the marrow.

In a recent study from Orlic et al[18], transplantation of selected cells from the bone marrow in an infarctional zone in mice's heart, with encouraging results about the differentiation and the functional result was reported. The researchers mentioned that 27 days after the infusion in the infarctional zone of cells from the bone marrow, they observed a well-differentiated myocardial tissue, a 68% mortality reduction, a 40% reduction of the infarct size, a 26% reduction of left ventricular dilatation, a 70% decrease of the diastolic tension, and a gradual improvement of ejection fraction. The formation of a vascular plexus connected to the vessels of the peri-infarctional zone was also discovered. The researchers attribute this successful activation of the grafted cells to the influence of the cytokines produced in the infarctional zone.

In another study with cell transplantation from the bone marrow into hamsters with dilated cardiomyopathy[19], the cells from the bone marrow proved less effective than the myoblasts.

Clinical trials concerning bone marrow cells transplantation in humans after myocardial infarction are in progress.

In the TOPCARE-AMI[20] trial, the researchers performed intracoronary infusion of stem cells in 20 patients with myocardial infarction and a patent culprit artery 4.3 ± 1.5 days after the infarction. These patients compared with the control group, presented during a 4-months follow-up, a statistically significant improvement of left ventricular ejection fraction, wall motion improvement of the infractional area and decrease of left ventricular end-diastolic volume. A great increase of coronary flow reserve of the culprit artery was detected. Viability study of the infracted area, with Positron Emission Tomography (PET) showed significant improvement of the infarcted area. Neither inflammatory reactions, nor arrhythmias were detected.

In another study of Strauer et al[21], 10 patients suffered from myocardial infarction underwent intracoronary infusion of bone marrow cells in the culprit artery, during a coronary artery angioplasty procedure. Control group consisted of 10 patients with similar clinical presentation, in whom no cells transplantation was performed. After a 3-months follow-up a statistically significant reduction of the size of the infarcted area and an increase in the systolic motion velocity of the infarcted wall compared with the control group, were detected. Further examinations (dobutamine stress echocardiography, radionuclide ventriculography and catheterization) that were performed to the group of patients who underwent cells transplantation, showed significant increase of stroke volume, reduction of end-systolic volume, improvement of left ventricular contractility and increase of blood flow in the infarcted area. The researchers concluded that bone marrow cells transplantation probably inducts a cardiac function improvement through regeneration and angiogenesis in the infarct tissue.

Stamm et al[22], performed implantation of bone marrow AC 133^+ cells in the myocardial infarcted region in 9 patients during a coronary artery by pass surgery. Three to 9 months later, all patients were alive, left ventricular systolic function was improved in 4 patients and blood flow in the infarcted region was significantly increased in 5 patients. The researchers concluded that cell transplantation is safe, and can improve myocardial function via angiogenesis and improvement of the blood supply in the infarcted region.

The same conclusions were published by Tse et al[23], who tried intramyocardial implantation of bone marrow cells via a percutaneous catheter, in the infarcted region of 8 patients.

The duration of the favorable effects of bone marrow transplantation in the myocardial function was examined in the study of Galinanes et al[24]. The study included 14 patients who underwent bone marrow cells implantation in the myocardial scar tissue during a coronary artery by pass surgery, after a myocardial infarction. In this study, the significant improvement of the ejection fraction that was detected 4 week after the cells implantation persisted and after a 10-months follow up period.

From the data published until now, it seems that the cells from the bone marrow are superior to the myoblasts especially concerning their flexibility of differentiation into myocardial tissue and their effectiveness. On the contrary myoblasts are easier to be obtained.

c) Smooth muscle cell transplantation.

The smooth muscle cell transplantation has the advantage of easy implant receiving and certainly the avoidance of immunosuppression.

In a study[25] gastric smooth muscle cells were implanted into hamsters' hearts, with previous myocardial infraction. Eight weeks later, smooth muscle cells and a degree of angiogenesis, probably due to vascular endothelial growth factor (VEGF) secretion from the grafted cells, were detected at the scar zone. It has also been discovered that the left ventricular dilatation was eliminated and its function was improved. However, the transplanted tissue didn't create any electromechanical conjunction with the surrounded myocardial tissue and so its contraction was asynchronous.

d) Fetal cardiomyocytes transplantation

The incapability of the implant to be connected with the surrounded myocardial tissue and the asynchronous systolic activity seem to be solved, at least in theory, with the fetal cardiomyocytes transplantation, where the differentiation problem doesn't exist. The possibility of fetal cardiomyocytes transplantation firstly appeared in a trial[26] where the researchers discovered the formation of intercalated discs between grafted and host cardiomyocytes. This grafted tissue (in hamsters and dogs) aligned completely to the existed myocardium and survived for 10 weeks[27].

Other researchers implanted fetal cardiomyocytes groups into the subcutaneous tissue of adult mice[28]. These cells not only survived but they also formed myocardial tissue. Later, this tissue was removed from the primary implantation area and transplanted into the scar tissue of the myocardium. The transplantation of this tissue leaded to an increase of the thickness of the scar tissue, reduction of the surface of the scar, reduction of the left ventricular dilatation and improvement of myocardial function[29].

In another study[30], left ventricular function was echocardiographically assessed in mice to which cardiomyocytes have transplanted into the myocardial infarction scar zone. A month after the transplantation, echocardiography and Doppler study, indicated an increase in ejection fraction and in the cardiac output, and also a reduction of the surface of the infarct region according to the results of the examination before the cell transplantation.

The results of the studies mentioned above are estimated as especially satisfactory and hopeful. But the clinical application of this method faces serious problems, as, moral problems according to the use of fetal cells, and also the necessity of immunosupresion treatment on a chronic basis. An additional, serious problem is the noticed decrease of the number of the live grafted cells, as the time passed despite the administration of cyclosporine, in an observation period of 20 weeks[31].

Parallel to the efforts for fetal cardiomyocytes transplantation, the research about the possibilities of fetal stem cardiomyocytes transplantation that are supposed to come either from blastocyst from vitro fertilization or from the cloning method are at a primary stage. It is widely known that the morale and legal problems haven't been solved yet. Alternative methods suggested are, receiver's cell reprogramming in order to return in a fetal primitive stage and extraction of animals cells that can be genetically modified.

e) Autologous cardiomyocytes transplantation

Autologous cardiomyocytes transplantation, (receiving of cells from the heart of the patient himself and their transplantation into the scar zone), is a method that avoids the restrictions of the previous method; necessity of immunosupresion because of the danger of acute or chronic rejection, and also the moral hesitations for the fetal cells use.

In a study[32], researchers caused a scar in mice's myocardium. After the biopsy, they took cells from the atrial myocardium and transplanted them in the scar zone. This had as a result, the decrease of the

scar extension and of the left ventricular dilation and also the improvement of the myocardial function. The non-achievement of a normal alignment of these cells to the host cells at the scar tissue was attributed to the non-satisfactory contact of the transplant to the host tissue during the transplantation. As expected, there were no indications of rejecting the transplant.

Other researchers[33] caused myocardial infarction to pigs, by closing the left anterior descending artery. Percutaneous biopsy was performed in every animal afterwards and myocardial cells were obtained from the intraventricular septum. Four weeks later, the cells that had been culture, were implanted in the infraction area of the animal from which they had been received. The animals that underwent cells transplantation presented improvement of the left ventricular wall motion and of the cardiac output in comparison with the animals of the control group. No animal presented indications of rejection of the implanted cells. The encouraging results of the above researches indicate that the autologous cardiomyocytes transplantation is an also promising method of the cellular treatment of ischaemic cardiomyopathy.

4. Gene therapy

The experimental success of gene intervention and modification of inherited diseases leaded to the question if the gene modification of the suffering cellular functions of the myocardial cell of a failing heart can improve the heart function.

In an experimental study del Monte et al,[34] modified in vitro, using genes transferred with adenoviruses, the pumps of the cellular calcium at the sarcoplasmic reticulum (phospholambane-CERCA 2a), in myocytes of people suffering from heart failure. The study indicated restoration of the calcium distribution. The same researchers, in an in vivo research [35] performed the same gene interference in mice's hearts with systolic and diastolic function disorders caused by overload pressure, noticed a restoration of the heart in both parameters.

Other researchers are experimented in the gene modification of other myocyte functions such as the b receptors function[36], the apoptosis etc.

In a remarkably interesting study by Suzuki et al[37], an effort was made of replacing scar tissue, in mice's myocardium, with transplantation of myoblasts genetically modified in order to produce vascular endothelial growth factor (VEGF). The results of the research showed that the combined transplantation (myoblasts with VEGF) was significantly superior in decreasing the infraction extension and in the improving the left ventricular function compared with the simple myoblasts transplantation and compared with the control group, where there was no transplantation. Two weeks later, an increased angiogenesis and satisfying differentiated muscle tissue in the scar area was found. Also, no tumor formation was found.

Certainly, though the first results are satisfying, many questions that must be solved still exist about the gene treatment and the safety of the method regarding oncogenesis is still questionable.

Conclusion

The most established and effective method of heart failure is heart transplantation. The method has many problems in its application and the most important is the deficiency in transplants. These problems combined to the increase of heart failure incidence impose the search for new treatment methods.

Cell therapy is a very promising method. The experimental data from many of the methods mentioned above are encouraging. Of course they deal with heart failure due to ischaemia, since this is the simplest experimental model of heart failure. But until the era of clinical application of this method will arrive, there are many questions that need to be answered.

The first question concerns cell implantation. Until now there are suggestions for implantation into the epicardium during surgery, the injection of cells, the intracardiac implantation through a percutaneous catheter and recently the intracoronary infusion of cells[38]. The implantation method is very important because it concerns the cell survival, their sufficient dispersion, and also the possible damage in the myocardium with the risk of arrhythmiogenesis.

The possible implications of this method must be searched also. Apart from the possible heart complications, from the development in the heart of a tissue that is not completely differentiated and aligned to the existed normal myocardial tissue, the implantation in vitro of multiplied and possible genetically modified host cells has the risk of oncogenesis.

Finally, it must be checked if this method can be applied to other forms of heart failure, such as dilated cardiomyopathy.

The research continues intensively all over the world. Many of the questions mentioned above will be answered. As we have already mentioned the first myoblasts transplantation has been applied to a heart failure patient with good results. The moral objections to the use of fetal tissues have caused serious dilemmas all over the world, but as it seems, the governments of the West World show the tension to move on to a gradual release of the research, with many restrictions. As a conclusion, cellular cardiomyoplasty – a satisfactory term suggested for the method – may be soon a clinical accepted method of radical treatment of heart failure.

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