Cellular cardiomyoplasty: development of a technique to culture human myoblasts for clinical transplantation

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Abstract

Some recent studies have demonstrated that epicardial injection of autologous myoblasts, obtained from satellite cells of skeletal muscle, in association to coronary artery bypass graft surgery (CABG) in patients with decreased left ventricular function secondary to ischaemic disease could be of some utility to get a better recovery of ventricular function due to the ability of these cells to grow and generate new muscle fibers over the previous fibrotic scar. The aims are the setting up of a process for the collection of the cellular cardiomyoplasty in samples of multiorganic donations and to carry out this technique in the same surgical moment as the revascularisation is performed in two patients. For this purpose we obtained muscle through biopsy of 15 human multiorgan donors and of two patients. Separation of fatty tissue, minced, and further digestion with collagenase type I (1.5 mgr/ml/2 gr by weight) and trypsin 1x. Filtration of the cellular suspension, centrifugation and sowing of this suspension in culture medium, with 20% of human serum. Culture for three weeks until obtainment of between 200–300 million cells. Immunohistochemistry and flow cytometry for the identification of the myoblasts was carried out. The results were obtained through flow cytometry, using CD56 as an indicator of the presence of myoblasts, between 70 and 80% of these types of cells were obtained after three weeks of culture. By immunohistochemistry analyses, different markers were analyzed: desmin and myogenin. The results indicated the presence of a great number of positive cells with these markers, possibly myoblasts. Skeletal myoblast implant was not associated with adverse effects. The culture of autologous myoblasts is a rapid and simple technique where after three weeks of culture a great number of cells for implantation are obtained. In patients with old myocardial infarction, treatment with skeletal myoblast in conjunction with coronary artery bypass is safe and feasible. and it is easy to obtain myoblasts from muscle tissue for transplant into patients.

Introduction

Cardiovascular disease continues to be the first cause of death in Spain, being more than 40% of total deaths in our country. In the eighties, the considerable increase in the use of fibrinolytic treatments, heparin, aspirin and coronary angioplasty grew parallel to better short
and long term survival after a myocardial infarct.

Nevertheless, the efficacy of the therapies available for advanced cardiac illness is extremely limited.

Cardiac transplant as an alternative to the medical therapies has also important limitations due to the lack of enough donors and the rejection and immunosuppression therapy risks. Moreover left ventricular assist devices (LVAD) or dynamic cardiomyoplasty are again limited procedures and with a relatively high morbidity.

An important aspect of physiopathology of ischemic cardiomyopathy and secondary cardiac failure is the irreversible destruction of cardiomyocytes. This associated to the absence of precursor cells makes the myocardial recovery after an infarct impossible transforming the normal heart into a dilated one in an effort to compensate the volume and pressure increases.

Cell transplantation has been examined in many recent studies as a potential method for repairing a damaged heart (Kedes et al. 1993; Li et al. 1998). The loss of cardiomyocytes after a myocardial infarction frequently results in thinning and dilatation of the resulting scar that can contribute to the associated ventricular dysfunction. Introducing viable cardiomyocytes into the scar region may modify the remodeling process and prevent heart failure.

The first contractile cells used were fetal derived cardiomyocytes. But, because of the problems that their use leads to, such as ethical aspects, immunological questions or difficulties as to their obtention, has made their clinical application simply a hope for the future. For this reason the autologous skeletal myoblasts have become the objective of several investigational lines (Hutcheson et al. 2000).

Successful transplantation of cultured cardiomyocytes into normal myocardium was reported in the early 1990s (Koh et al. 1993; Soopaa et al. 1994).

Cellular cardiomyoplasty using myoblasts obtained from satellite muscle cells attempts to repair the non-contractile myocardial tissue being a complement to CABG surgery allowing for a greater recovery of the cardiac function (Chiu et al. 1996; Atkins et al. 1999; Sakai et al. 1999; Menasche et al. 2001; Rajnoch et al. 2001). The performance of cellular cardiomyoplasty in the same surgical act as that of revascularisation does not increase the morbidity of the surgical procedure (Herreros et al. 2003).

The main objectives of our study can be summarized as follows: The setting up of the technique for autologous skeletal myoblast culture in samples of multiorganic donations and transplantation of autologous skeletal myoblasts directly into the myocardium in two patients.

Materials and methods

Biopsies

The fine tuning of the procedure for cellular culture was done using muscle tissue samples obtained from the vastus lateralis, as residues from multiorganic donations (n = 15) obtained with informed consent. The multiorganic donations were carried out in the operating theatre, in sterile conditions, which are maintained throughout the process of manipulation and transport.

At the same time, to perform the clinical trial, samples of tissue were obtained from the vastus lateralis in two patients for autologous culture with informal consent obtained: one 53 year old man, hypertense, hypercholesterolemia and ex-smoker. He had ischemic cardiomyopathy from 1998 with an extensive anterior infarct and as a result severe left ventricular dysfunction and consequently the ejection fraction (EF) was 20%. The other patient was a 66-year-old male patient with a history of smoking as the only cardiovascular risk factor. His ischemic cardiomyopathy was first diagnosed in 1982 when he had a posterior infarct.

Transport solutions

From the moment of dissection to the moment of reception in the laboratory, that is to say during the period of cold ischemia, the tissue was maintained at 4 °C in PBS. The tissue remained in these conditions until it was transported to the culture laboratory (3–6 h).

Cellular culture and determination of the purity and viability of the myoblasts

Three weeks (before surgery, in the case of the samples of the two patients), a muscle biopsy