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Transcutaneous intramyocardial injection technique for implantation ofbone marrow derived stem cells in children with idiopathic dilated cardiomyopathy

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ABSTRACT

An increasing understanding of the nature and processes of idiopathic dilated cardiomyopathy in children, as well as the limited treatment options have led several researchers to use stem cell transplantation in management of these patients. Study results suggest that homing of transplanted cells varies significantly using different techniques for their delivery to the target area. We will describe in detail the technique for transdermal intramyocardial implantation of bone marrow derived progenitor cells. This innovative technique if combined with ultrasound monitoring offers the possibility for delivery of stem cells right into the target area in a safe and effective way.

Keywords: Abattoir effluents, *Staphylococcus aureus*, Antibiotic susceptibility, Abakaliki

INTRODUCTION

Dilated cardiomyopathy is a serious problem in pediatric cardiology. Despite the relatively low incidence 0.57 to 2.6 per 100,000 children, the mortality rate is high. One third of patients die within the first year after diagnosis [1, 2]. Up to 40% of them are defined as idiopathic dilated cardiomyopathy (IDCM) characterized by ventricular dilatation and systolic dysfunction of unknown cause. The prognosis is poor in pediatric patients and survival appears to be related to the

degree of systolic dysfunction. Conventional medical therapy often provides a short term relief of symptoms but does not improve the outcome of the disease. However, the recent clinical studies [3, 4] have suggested bone marrow-derived autologous mononuclear cells or circulating progenitor cells [5, 6] as a promising therapy option for these patients. However the most effective and safest way for delivery of these cells to the target area still needs to be established.

In collaboration with The Latvian Center of Cardiology, Pauls Stradins Clinical University Hospital and Cell Transplantation Center,we have have been using the technique of transcutaneous intramyocardial stem cell transplantation since May 2009. The Ethics Commission has granted permission to perform the procedures described in this article.

MATERIALS AND METHODS

Preparation of Bone Marrow Aspirate

5 to 30 ml of bone marrow was aspirated from iliac crest, isolating 17 to 122 million BMCs. Samples for flow cytometry were taken from 15 ml of mononuclear cell fraction and prepared for transplantation. Preparation of the isolated cells was carried out using Stem-kitTM reagents (Cat. Nr. IM3630; Beckman Coulter), that contained CD34-PE, CD45-FITCm isotype control, 7AAD (viability dye) and Stem-Count Fluorospheres. FACS analysis was performed on FC-500 (Beckman Coulter) and analyzed using CXP software. Each measurement contained at least 50,000 events. Maximum number of events was 100,000. Obtained numbers of cells/µl were calculated for a total number of CD34+ cells within transplantation material. Measurements with less than 50,000 events were excluded from the study.

Technique for Delivery

The procedure was performed under general anesthesia in the operating theatre, positioning a patient horizontally on the back. We used the 0.95 mm × 220 mm OptiMed CHIBA needle. Similarly to the technique of pericardial puncture we used the transcutaneuos subxiphoid approach, poking through the skin (dot (1) in Fig 1A) followed by gentle advancing toward the cardiac silhouette until a slight negative pressure was felt, searching for the *apex cordis* (dot (2) in Fig 1A). When the apex cordis was reached, under echocardiographical control (Hewlett-Packard SONOS 4500) we performedperformed an injection of 1 ml of cell suspension, subsequently followed by a second dose of 1 ml suspension with1-2 min interval in between. A rationale to divide the injectable volume into two doses was based on concerns about a potential risk of barotrauma in myocardial tissues if the volume of a single dose would be too high.

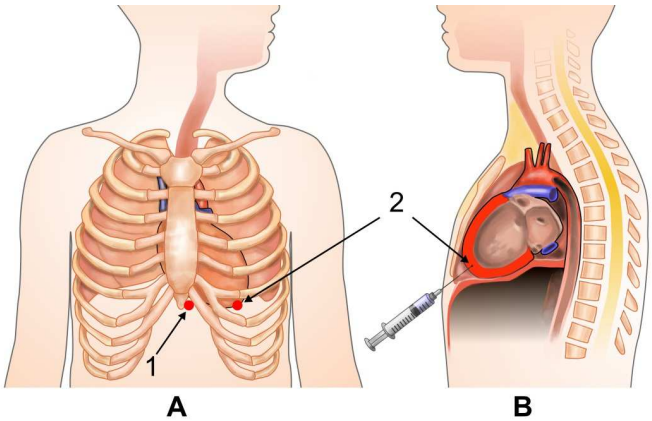

Figure 1

(A) Schematic positioning of transcutaneous (1) and intramyocardial (2) punction.

(B) Injection of cell suspension into the *apex cordis.*

Injection of cell suspension is controlled throughout the procedure via the US monitoring. The most challenging step of the procedure is simultaneous movement of the US probe and the needle towards the *apex cordis.*A special attention has to be paid to risks related to potential penetration of the ultra-thin wall of the left ventricle.

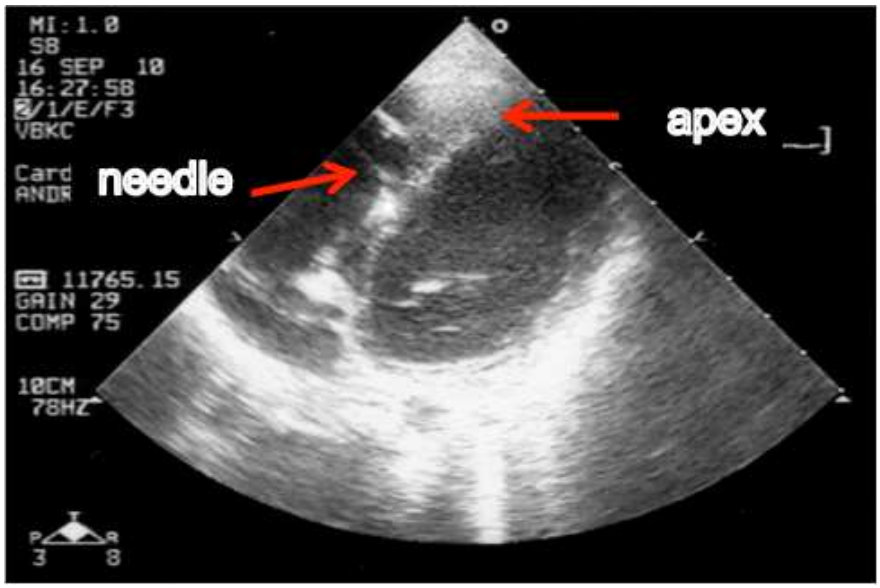

Figure 2
Echocardiographical visualization oftranscutaneous intramyocardial injection of the stem cells

Shortly after injection of the cell suspension, an additional US check should be done to visually check the immediate results of the procedure. While learning the echocardiographicallyguided technique, we suggest radiographic visualization for further control for the short-term effects of the procedure. Analgesia is administered as needed.

DISCUSSION

The management of idiopathic dilated cardiomyopathy in children is mainly conservative, and includes medication of the underlying heart failure with diuretics, angiotensin-converting enzyme (ACE) inhibitors, and beta-blockers. Available data suggest that ACE inhibitors are the only group of pharmaceutical agents prolonging survival, whereas others appear to provide just symptomatic relief. Cardiac transplantation is widely regarded as the end-stage therapy for these patients. However, the lack of donors is an important limitation of this procedure. Furthermore, a significant part of these patients do not live long enough to undergo heart transplantation because of the often delayed diagnosis. All of the data mentioned above may suggest there is a critical need for brand new treatment options, providing new prospects for these critically ill patients.

Stem cell research has been accelerating during the last few years. As a result of numerous studies performed during the last decade, a significant progress in understanding of clinical effects provided by the stem cell therapy has been made. However, the vast majority of these studies have been done in the adult population whereas there are relatively few of them devoted to the possible implementation of the stem cell therapy in children. Furthermore, the data from studies in adults

usually cannot be directly extrapolated for the use in children because most of the suggested techniques used are not applicable to the children for both medical and technical reasons.
Cardiology is one of the areas in which the use of stem cells could play a critical role, aiming at the repair of compromised myocardium and therefore improving the prospects for severely ill patients. Induction of myocardial cell recovery poses a potential to significantly improve survival and the quality of life in patients with both congenital and acquired heart conditions in cases where conventional therapy appears not to be effective, failing to provide desired results.
Several methods have been described for the delivery of autologous bone marrow stem cells in heart disease patients: subcutaneous [7], intravenous or intracoronary [8-13], andintramyocardial via infusions during cardiac bypass surgery[14] or via transendocardial injections[15]. Intramyocardial and intracoronary route of administration appear to be the most widely used techniques.
In adults, the use of stem cell therapy in cardiology appears to be mainly associated with its use in the management of ischemic heart disease, myocardial infarction in particular. In this respect there are thousands of patients worldwide who benefited from the stem cell therapy. As to children, the main target group in this therapeutic area appears to be the patients with idiopathic dilated cardiomyopathy being an important cause for chronic congestive cardiac failure. However, many questions still remain unanswered, particularly regarding the choice of appropriate cell type, dose, method of administration and the most appropriate timing for stem cell transplantation.
It appears important to emphasize that there is a significant difference between introcoronary and intramyocardial injection of stem cell substrate if to compare the number of introduced stem cells deposed nearby or in the target tissues shortly after the procedure. In fact, shortly after the intracoronary injection, most of the introduced cells get into the bloodstream and only a small part of them remain in the coronary blood vessels, the rest find their path to various organs and tissues by different homing routes.

Some researchers have reported on a progressive decrease in the number of detectable stem cells in the target area shortly after the intracoronary infusion. During the period from one hour to four days after the infusion of radioactive labeled stem cells theirconcentrationin myocardial tissues decreased from the mean of 6.9+/-4.7%to 2+/-1% within 3 to 4 days after the procedure[9-11].Since just a small fraction (<10%) of the injected cells, which are supposed to be functionally active, are retained in the target tissues for a prolonged period of time due to the biodistribution, it is unlikely they induce remarkable functional effects[10].

In clinical practice, intracoronary stem cell infusion can be performed using different types of cardiac catheterization techniques. Unfortunately, in pediatric patients these methods are often associated with high risks to damage coronary vessels followed by the formation of blood clots (1.9%) and/or myocardial ischemia [11].Therefore, this way of administration cannot be supposed as the method of choice in children, especially at an early age, taking into account the age related biological features and available technologies.
As suggested by some authors, intravenous application would require many circulation passages to enable infused cells to come into contact with the area of interest. Throughout this long circulation and recirculation time, homing of cells to other organs could considerably reduce the number of cells dedicated to cell repair in the tissues supposed as an initial target zone [11,14].
Intramyocardial stem celltransplantationprovides a number of the long-term effects on the target tissues, i.e.the heart muscle. In particular, it may be expected that such intervention stimulates myocardial regeneration via stem cell transformation and generation of new cardiomyocites[16], revascularisation[17], stimulation of myocite contractility due to paracrine effects [18], and positive impact of apoptotic cells on the myocardial remodelling[19]. Experimental research data suggest

that chemokines, cytokines and growth factors deposited in the intercellular space may potentially be involved in cardiac repair.
Intramyocardial administration of stem cells in adults is usually performed during the open chest surgery via injection of the stem cell substrate right into the area of myocardial ischemia, often combining with coronary bypass grafting [14,21].
An alternative route for cardiac stem cell transplantation is transendocardial catheter injection, offering intramyocardial cell delivery similar to the surgical approach with being less invasive at the same time [14,15].

Research data suggests that the functional improvement occurs in less than 72 hours after intramyocardial injection of bone-marrow derived stem cells [18]. Together with our data, these data support the hypothesis of the role of paracrine effects role in ensuring positive dynamics, to a lesser extent linking it with stem cell transformation to cardiomyocites which in such a short time frame is less feasible.
Unfortunately, none of the methods for cardiac stem cell transplantation mentioned above is applicable in children with dilated cardiomyopathy. In accordance with our experience, the majority of patients at the time of diagnosis experience clinically significant heart failure (NYHA III-IV), creating additional risks for eventual open-chest surgery followed by injection of stem cells into the heart muscle under visual control. Transendocardial injection as an alternative also remains questionable due to anatomical (small vessel diameter) and technical (availability of appropriate catheters) limitations. In fact, extremely thin walls of the ventricles pose significant risks for their penetration during the transendocardial injection. Often the expected benefit risk balance is assessed as negative, taking into account the general condition of the patient and high risks of periprocedural complications. Due to the reasons above, we have chosen a relatively safe and effective method for administration of stem cells right into the target tissues, using transcutaneous intramyocardial input under echocardiographical monitoring[22-24].
All the mentioned considerations appear to justify the method of administration of stem cell substrate via injections just into the target tissues rather than in the regional blood vessels or any other distant locations of the body.

CONCLUSION

In summary, any technique used for transplantation of stem cells in children with idiopathic dilated cardiomyopathy has to satisfy the condition of acceptable benefit/risk ratio, aiming at maximum safety and the evidence based efficacy. Technique of transcutaneous intramyocardial transplantation under echocardiographicalcontrol offers the possibility of precise injection into the target area and real-time surveillance of the procedure. This technique has all the potential advantages of minimally invasive surgery and opens a new prospective for management of critically ill children with idiopathic dilated cardiomyopathy at very early age, regardless of the degree of underlying heart failure.

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