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INTRAMYOCARDIAL TRANSPLANTATION OF PERIPHERAL PROGENITOR CELLS IN PATIENTS WITH ISCHEMIC CARDIOMYOPATHY AND CARRIAGE OF THE 4G/5G POLYMORPHISM OF PLASMINOGEN ACTIVATOR INHIBITOR TYPE 1 GENE (PAI-1)

**Zaporozhan V. N., Bajora I. I., Karpenko I. I., Kozlov V. P.,
Naidenko N. V., Kozlov I. V., Zukow W.**

**Odessa National Medical University, Odessa, Ukraine
Kazimierz Wielki University, Bydgoszcz, Poland**

Abstract. The article reflects the influence of 4G/5G polymorphism of the plasminogen activator inhibitor type 1 gene (PAI-1) on the progression of congestive heart failure (CHF) and ischemic cardiomyopathy (ICMP). Indications for intramyocardial transplantation of peripheral blood stem cells (PBSC), depending on the carriage of different PAI-1 alleles were determined. The study comprises 45 patients with ICMP, who are resistant to standard medical therapy and for whom surgical treatments are contraindicated (or were not effective). The patients were divided into 2 groups: in group 1 (n =15) intramyocardial PBSC transplantation was performed, group 2 (n=30) underwent a standard drug therapy. 4G/5G polymorphism PAI-1 was determined for all the participants of the study.

The results showed that the carriage of 4G/4G polymorphism PAI-1 is a predictor of severe progression of congestive heart failure and a risk factor for the development of ICMP. It is recommended to perform intramyocardial PBSC transplantation to the patients of the study as early as possible.

Key words: intramyocardial transplantation; progenitor cells; patients; ischemic cardiomyopathy; carriage; 4g/5g polymorfism; plasminogen activator; inhibitor type 1 gene; pai-1.

Cardio-vascular diseases and pathologies are steadily increasing in the world. A significant proportion is accounted for ischemic heart failure. In the basis of the pathogenesis of this disease lies the old myocardial infarction or the influence of chronic violations of the coronary circulation on the homeostasis of the heart muscle, which leads to dilation of the heart cavities, cellular hypertrophy and remodeling of the extracellular matrix [1]. Also, there are more than 150 genes, the polymorphic variants of those are associated with the development of cardio-vascular diseases [2]. The allelic variants of plasminogen activator inhibitor PAI-1 [3] are of particular interest. This cytokine takes a key role not only in the system of fibrinolysis, inflammatory reactions, but also in the pathogenesis of atherosclerotic coronary artery disease, myocardial remodeling processes in the presence of ischemic lesions and cell proliferation.

Traditional medical and surgical treatments of acute or congestive heart failure (CHF) do not always give a positive result, which leads to a constant search for alternative ways of correcting such conditions [4].

The rapid development of biotechnology, providing for the use of stem cells has created a fundamental platform for their introduction into clinical practice [5-7].

At present the technologies for derivation of progenitor cells from different sources have been developed: i.e. from bone marrow, adipose tissue, umbilical cord blood [8, 9]. There are certain advantages and disadvantages of each method of derivation of the cells-precursors. However, the use of peripheral blood for obtaining the therapeutic cell suspension containing stem cells has indisputable advantages among most of the existing methods: safety for the patient, no need for cultivation, low risk of contamination of donor cells, the

possibility of the sampling of the primary cytological material in the manipulation room.

The promising method for the regeneration of damaged vasculature is the method of application of autologous hematopoietic stem cells of peripheral blood (PBSC) which are capable of multilineage differentiation and self-regeneration [10]. It is also shown that PBSC are able to be differentiated into almost all tissues of the body, including cardiomyocytes [11].

The aim of our study was to investigate the efficiency and safety of intramyocardial PBSC transplantation and to determine the indications for transplantation, depending on the 5G/4G polymorphism of PAI-1 gene at the patients with ischemic cardiomyopathy (ICMP).

Materials and methods. The main criteria for the inclusion of patients in the study were: men and women aged 45-75 years, suffering from ICMP III-IV NYHA, who are resistant to standard medical therapy and for whom surgical treatment (coronary artery bypass grafting or stenting) is contraindicated (or was not effective).

The main exclusion criteria were: the passed myocardial infarction within 3 months, the operation of stenting or coronary artery bypass surgery within 3 months, active oncopathology within 5 years, and pregnancy.

A total of 45 patients were included. All participants of the study were divided into 2 groups.

The first group (n=15) included the patients who received standard medical therapy and who underwent a surgery of intramyocardial PBCS transplantation.

The second control group (n=30) included the patients who received only standard medical therapy.

All patients with atrial fibrillation were given anticoagulant therapy. The patients with low risk of thrombotic complications under the scale CHADS2-

VASc took Rivaroxaban 20 mg per day. Patients with atrial fibrillation and the presence of such risk factors as III-IV degree of spontaneous contrast, reduced blood flow velocity in the left atrial appendage, the presence of II-III degree of mitral valve insufficiency, reduced local and global contractile function of the left atrium and its appendage were given a dose of warfarin (2.5-9 mg) a day under control of the international normalized ratio.

4G/5G polymorphism of PAI-I gene was determined by means of polymerase chain reaction for all patients of the study [12].

In order the progenitor cells leave the bone marrow and come into the peripheral blood, the patients of the first group were given a single subcutaneous injection of granulocyte colony-stimulating factor (G-CSF) filgrastim at a dose of 48 million units prior to the procedure of the sampling of primary cytological material (PCM).

Sampling of PCM was carried out by leukapheresis on the blood cell separator Fresenius COM.TEC, set C4Y and the program PBSC-lym, under the constant supervision of the general condition of the patient and cardiac monitoring. As a vascular access the catheterization of two peripheral veins – the central and peripheral veins - was applied.

The processing of leukapheresis liquid was carried out under the conditions of a high dose chemotherapy and bone marrow transplantation block in laminar flow microbiological safety cabinet II.

The isolation of mononuclear cells was carried out by gradient centrifugation method recommended in literature [13]. To create a density gradient a sterile polysaccharose and diatrizoate solution with density gradient 1.077 g/ml (Sigma-Aldrich, UK) was used. The resulting cell suspension for transplantation was concentrated in 8 ml solution of sodium chloride with concentration of 0.9%.

Before the transportation of cell transplants to the operating room the number of mononuclear cells was calculated by means of optical microscopy in

Goryaev's chamber. The viability of cells which had to be not less than 92% was determined by staining with 0,4% tripan blue solution by standard technique.

The intramyocardial transplantation of progenitor cells was performed after creating elektromechanical maps of left ventricular (LV) in the hibernating myocardium zones by means of NOGA.XP Navigation System and catheter MyoStar. The position of the catheter in the left ventricle cavity was additionally monitored radiologically and by means of intracardiac ultrasound.

The clinical condition and dynamic of the disease was assessed by the control investigations of the patients 1, 6 and 12 months after the transplantation, which included: general clinical research, electromechanical mapping of the left ventricle by means of the reference electrode, echocardiography, electrocardiogram and exercise tolerance test (ETT).

The exercise tolerance was measured by means of 6-minute walking test proposed by G.E.Gendlin and his colleagues. The quality of life was assessed by the results of the Minnesota Quality of Life Questionnaire of the patients with CHF. Echocardiography was performed in accordance with recommendations of the American Association for echocardiography and by standard techniques.

The comparison of polymorphisms frequency of PAI-1 gene at the patients with ischemic cardiomyopathy was performed with a frequency of occurrence in the normal population on the basis of previous studies [14].

Results. In the study of polymorphism of PAI-1 gene it was noted that the patients with ICMP in 57,7% (n=26) of cases were carriers of 4G/4G polymorphism; in 24,4% (n=11) of cases – carriers of 5G/4G polymorphism; in 17.7% (n=8) of the cases – carriers of 5G/5G polymorphism. In the normal healthy population the frequency of occurrence is the following: 4G/4G - 26%, 5G/4G - 40%, 5G/5G - 34%.

In comparison of the frequency of occurrence of polymorphism of gene PAI-1 at the patients with ICMP with the frequency of occurrence in healthy human population there is a significant difference ($p < 0,05$).

By the introduction of G-CSF the following side effects were determined: flu-like symptoms in 66.6% of cases (10 patients), increased shortness of breath - in 20% of cases (3 patients), increased complaints of stenocardia in 60% (9 patients).

For the separation procedure the catheterization of two peripheral veins in eight cases (53%) was applied, in the remaining cases the central vein was used for the venous access.

During the sampling of PCM only one patient noted the feeling of "pins and needles" on the skin of the lower limbs and the "hair" on the tip of his tongue. The adverse effect passed after the intravenous injection of calcium gluconate 9%.

All cell transplants after processing conform to the requirements of the protocol: they contained a minimum of 100 million cells per 1 ml, the viability was not lower than 92% and they were sterile according to the results of 8-day incubation at 22 °C and 32 °C under aerobic and anaerobic conditions.

All patients underwent an intramyocardial PBSC transplantation surgery satisfactorily. During and after the operation disturbances of conductivity and life-threatening arrhythmias were not determined. It took in average 112 ± 7 min to make the contractility LV's maps and identify areas of hibernating myocardium and about 51 ± 3 minutes for the implantation of cell suspensions. The average total operative time was 137 ± 5 min. 18 ± 3 points were selected for implantation and $0,3 \pm 0,1$ ml of cell suspension was introduced.

The postoperative period for all participants of the study passed without complications. The patients left the hospital after six days in average.

In the course of monitoring of the patients during the year the significant tendency towards improvement of cardiac pump function was determined in the

first group compared to the control one (Tab. 1). This was proved by the results of the electromechanical mapping of the left ventricle using the NOGA.XP navigation system (Fig. 1, 2) and echocardiography (Tab. 1).

Also, it was noted that the maximum effect developed after 6 months after transplantation.

Tab. 1. Dynamics of some indices of LV function and severity of CHF

| Data | LVEF (%) | | EDV (ml) | | 6 min walking test (m) | |
|--------------------------------------|-----------|----------|------------|-----------|------------------------|----------|
| | Gr. 1 | Gr.2 | Gr. 1 | Gr.2 | Gr. 1 | Gr.2 |
| The observation time | | | | | | |
| Prior to PBSC transplantation | 23,7±0,4 | 23,7±0,2 | 249,5±1,6 | 248,9±1,0 | 27,6±0,1 | 29,1±0,5 |
| 1 month after PBSC transplantation | 24,7±0,2 | 23,3±0,1 | 250,4±0,4 | 250,5±0,2 | 30,0±0,1 | 31,0±0,1 |
| 3 months after PBSC transplantation | 28,0±0,2* | 23,2±0,2 | 243,0±0,1* | 249,1±0,2 | 80,1±0,2* | 39,9±0,1 |
| 6 months after PBSC transplantation | 32,0±0,1* | 23,0±0,1 | 211,0±0,1* | 251,0±0,1 | 151,0±0,1* | 40,1±0,1 |
| 12 months after PBSC transplantation | 33,0±0,1* | 23,9±0,1 | 208,1±0,1* | 249,3±0,1 | 200,0±0,1* | 55,0±0,1 |

By "*" the reliably significant results are marked (p<0.05)

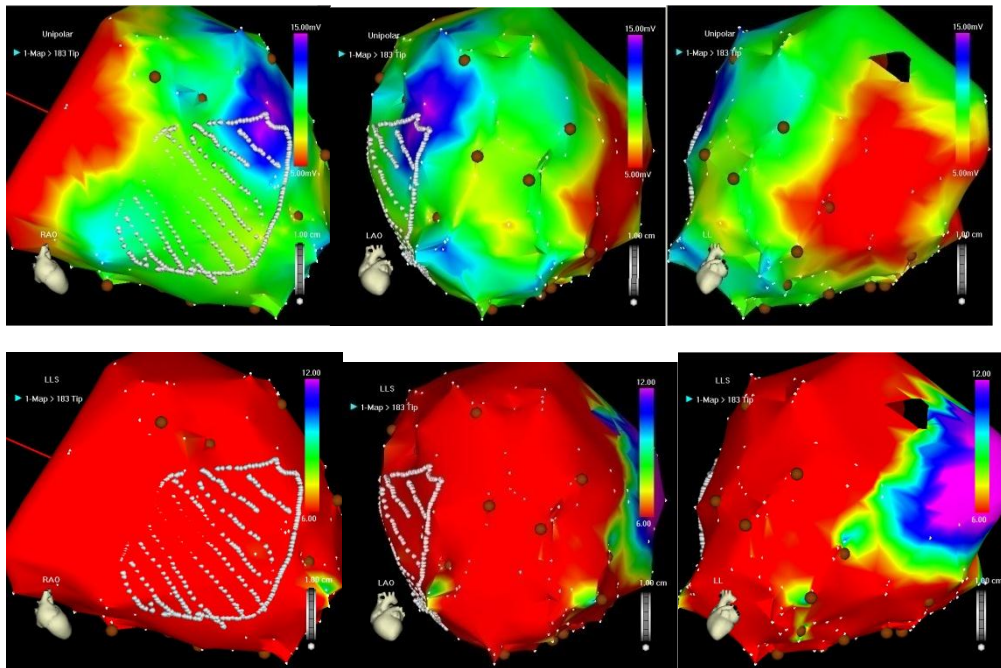


Fig. 1. Mapping of the left ventricle. At the top there is a unipolar voltage map, at the bottom - left ventricular contractility map. The red color on the voltage map means the absence of electric potential, on the contractility map – the absence of contraction. The points mark the site of implantation of PBCS.

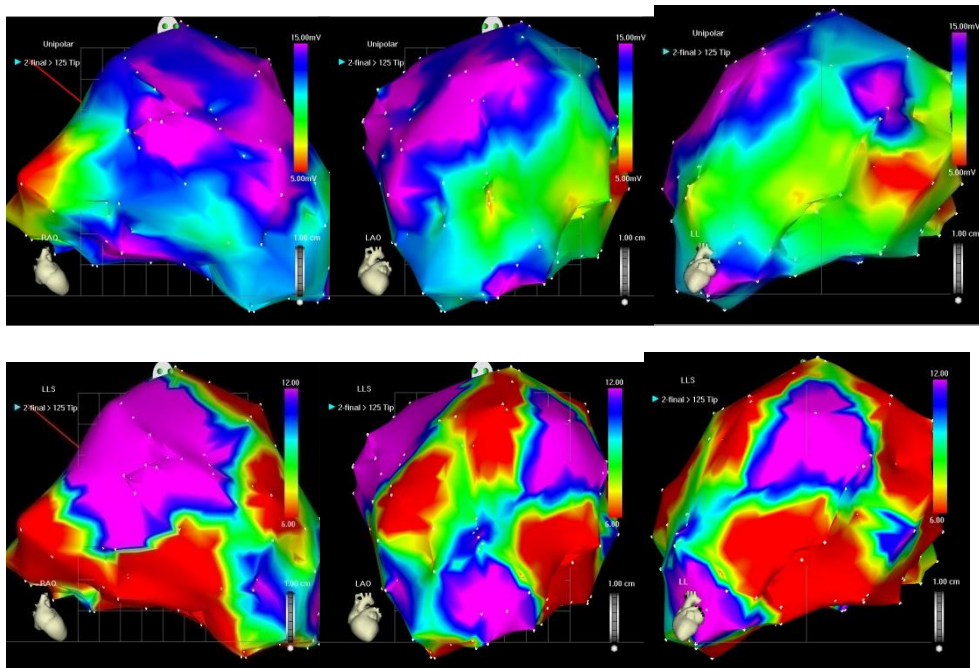


Fig. 2. Mapping of the left ventricle 12 months after PBSC transplantation. Changes in red towards purple mean the appearance of an electric potential (on the voltage map) and improved contractility (on the contractility LLS map). The significant improvement in conductivity and contractility is observed.

As a result, in the group where PBCS transplantation had been carried out, it was noted that the left ventricular ejection fraction increased by an average of $9,3 \pm 0,3\%$, EDV decreased by $41,4 \pm 0,1$ ml. According to echocardiography all patients reported improvement in local and global left ventricular contractility.

All patients of the first group underwent during the control investigations Holter ECG monitoring, which did not state the emergence of new arrhythmias.

Discussion of the results. This study confirms that the carriage of 4G/4G polymorphism of PAI-1 gene is a predictor of a more severe course of chronic myocardial ischemia. One can say that this polymorphism is a risk factor for myocardial remodeling of the left ventricle, the result of which is the development of ICMP. PBCS significantly improve the pumping function of the

left ventricle, as proved by the echocardiography dynamics and electromechanical re-mapping of the left ventricle. Despite of intramyocardial way of introduction of cell suspension, none of our patients showed life-threatening arrhythmias.

The isolation of PBSC using separator of blood cells is a preferred method, since it is a safe and low-traumatic procedure for the patient which does not require general anesthesia, as in case of isolation of bone marrow stem cells.

The use of NOGA.XP navigation system allows us to determine accurately the areas of hibernating or "sleeping" myocardium for the zone selection for PBSC implantation.

Based on the literature data and the results of our research we believe that it is advisable to perform PBSC transplantation for the patients who suffer from CHF or ICMP with low LVEF and are the carriers of 4G/4G polymorphism of PAI-1 gene as early as possible. This will improve their quality of life and reduce mortality.

Conclusions.

1. Carrying of 4G/4G polymorphism of PAI-1 gene is a predictor of severe chronic myocardial ischemia and risk factors for the development of ICMP;
2. Patients with chronic heart failure with carriage of 4G/4G polymorphism of PAI-1 and EF less than 25% are recommended to undergo intramyocardial transplantation of progenitor cells as early as possible in order to prevent the development of left ventricular remodeling.

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