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Research Article

The application of multipotent mesenchymal stromal cells with overexpression of the vegf 165 gene to correct coronary insufficiency

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Abstract

A new approach to the treatment of patients with coronary heart disease is based on the use of either pharmacological drugs, mainly to eliminate the symptoms of the disease, as well as the prevention of" terrible " complications (myocardial infarction), or a combination of pharmacological and surgical methods (stenting and/or coronary artery bypass grafting).

However, the use of surgical methods is not always possible (there are a number of absolute contraindications to coronary bypass surgery and / or the use of interventional technologies) or is limited, for example, by the small diameter of the main coronary arteries or their angulation, and vascular stenting is accompanied by a high risk of thrombosis (with multivessel lesions) and the formation of restenosis. The simultaneous use of MMSCs transfected with VEGF-165, accompanied by synergistic effects, makes it possible to link future prospects for therapeutic angiogenesis with the use of stem cells transfected with growth factor genes and the factors themselves that enhance the effect of gene-cell constructs.

Keywords: arrhythmia; pediatrics, tachycardia-Induced cardiomyopathy; heart failure; left ventricular dysfunction; ablation; supraventricular tachycardia, atrial tachycardia

Introduction

It is known that the development of heart diseases is associated with a genetically determined deficiency of HIF production by heart tissues. As a result of HIF deficiency, which is responsible for the initiation of transcription of a wide range of factors (more than 30) responsible for the restoration of p02 in tissues and cell adaptation to hypoxia, necrobiotic processes develop in the heart muscle in response to hypoxia, followed by the replacement of the defect with connective tissue and the formation of CHD. In this case, the absence of neoangiogenesis is the defining moment. Full-fledged angiogenesis requires the expression of the entire gene complex, and VEGF 225 expression is crucial. In a simplified form, the sequence of events is as follows: under hypoxic conditions, HIF-1a is expressed; and HIF-1b:. which, in turn, combine into a single peptide and cause the expression of a battery of genes. However, another feature of heart tissues is a reduced expression of the VEGF 225 gene. In other tissues, VEGF 225 expression leads to accumulation of VEGF peptides resulting from alternative splicing, angiopoietins, and growth factors. The latter ensure the survival and mobilization of endotheliocytes. their migration to the ischemic zone, proliferation and formation of vessels in the ischemic zone. [1, 5].

However, only every fourth patient with coronary artery stenosis develops collateral vessels during occlusions, which is probably due to genetic factors [2,77].

Arteriogenesis forms collateral vessels from non-functioning arteriolar junctions that allow blood flow to bypass the occlusion site. The most important stimulator of arteriogenesis is an increase in shear stress above the occlusion site, which promotes the expression of adhesion molecules by endothelial cells with subsequent accumulation of monocytes in the vessel wall. The latter secrete active RFS, of which the main regulators of arteriogenesis are fibroblast RFS (FGF), as well as PDGF, VEGF, and chemokines [3, 7].

Effective myogenesis in the myocardium and skeletal muscles is impossible without angiogenesis, and angiogenesis is impossible without myogenesis. It is SCS and progenitor cells that can potentially stimulate both processes

[4, 6]. The mechanisms of reparative action of SCS, obtained from an adult organism, include paracrine effects, associated with their secretory activity, differentiation of SCS into specific клетки tissue and vascular cells , and fusion with клетками tissue cells, which allows them to impart им new properties [1, 3, 4, 5]. However, the experimental data obtained are quite contradictory. Thus, the involvement of SC in the construction of new vessels by differentiation into EC has been demonstrated in several experimental studies using transplantation of labeled bone marrow cells [4, 7]. At the same time, neovascularization is stimulated by the introduction of SCS to a large extent due to their secretory activity. This is confirmed by the fact that an increase in the number of vessels in the myocardium of experimental animals was observed with the introduction of almost all types of cells used for cell therapy: hematopoietic, mesenchymal bone marrow cells, EC precursors (circulating and bone marrow) obtained from umbilical cord blood, and even skeletal myoblasts [2,3, 4, 5].

When studying the effects of co-cultivation of rat CSF with cells isolated from the heart of newborn rat pups [3, 4], it was found that CSF contributes to the formation of more complex (branching) and stable vascular structures by postnatal heart cells. This effect is due not only to the paracrine effects of CSF, but apparently to intercellular interactions, since when adding the CSF culture medium to cells isolated from the heart, a smaller effect was observed. At the same time, vascular structures formed by endothelial (CD-31+) heart cells were surrounded by CSFTS expressing the NG2 pericyte marker. The presence of cells carrying pericyte markers in the SCLT population was also demonstrated using flow cytofluorometry. It is possible that, in addition to the paracrine mechanisms of neovascularization stimulation, the latter can directly participate in the formation of blood vessels and their stabilization due to the pericyte cells present in them.

In addition, MMSC are well susceptible to transduction by adenoviral, lentiviral, retroviral, and adeno-associated viral vectors. Genetically modified cells that overexpress the VEGF gene secrete 10 times more of this factor than unmodified cells. Thus, MMSC represent a population of cells with a high degree of plasticity and proliferation intensity, significant angiogenic potential, due to a large extent to their ability to secrete many pro-angiogenic and anti-apoptotic factors, and can be considered as an effective cell vector for the transfer of therapeutic genes. With a sufficiently high content of this type of cells in the VT, relative safety and low injury rate of their production, MMSC are promising candidates for therapeutic angiogenesis.

The aim of the study was to evaluate the completeness of neoangiogenesis and the restoration of normal myocardial perfusion levels при after intramyocardial administration of MMSCs transfected with VEGF165 in ischemia modeling.

Materials and methods of research

The experiment was conducted on male Chinchilla rabbits weighing 2.8-3.2 kg and aged 1-1.2 years. Animals after premedication with atropine 0.04 mg / kg, to prevent edema of the tracheal mucosa, under thiopental anesthesia (intraperitoneally, 40 mg/kg) under artificial ventilation, underwent left sternotomy. In order to ensure incomplete occlusion of the anterior descending artery of the heart, its proximal segment was ligated on the mandrel, narrowing the lumen of the vessel by 80%. Animal group No. 1 (n=10) was given a saline solution that included all components except stem cells. Experimental group No.2 (n=10) received a single intramyocardial injection of MMSCs in the amount of 1.0 x106 cells per cm2, transfected with the VEGF-165 gene immediately after ligation.

MMSCs were obtained by explanting subcutaneous adipose tissue from the anterior abdominal wall in 10 laboratory animals. According to the cell isolation protocol:

1. Adipose tissue was obtained from the buttock area or anterior abdominal wall.

2. The tissue was crushed and washed twice from blood cells at 250 G in a phosphate buffer solution for 10 minutes.

3. The sample was placed in a 1% lyophilized crab pancreatic enzyme solution containing all types of collagenase.

4. The sample was incubated at 370C with periodic shaking for 120 minutes.

5. The enzyme was neutralized with an equal volume of DMEM medium containing 10% bovine bovine serum and filtered through a nylon filter with a pore size of 100 microns.

6. The sample was centrifuged at 1000 G, the precipitate was resuspended, seeded on culture plastic, and incubated for 3 weeks at 37 0C and 5%CO2 with a medium change every 3 days.

Transfection was performed with a plasmid with the VEGF165 gene (pWZL Blast VEGF165), regulated by the CMV promoter. Lipofection using Unifectin-56 (Unifect Group, Russia) was performed in MMSCs of confluent patients up to 60-70%. The ratio of Unifectin to plasmid is 12 U/mcg per 10 cm diameter Petri dish or 25 cm2 culture vial. The environment was changed the next day, then every 3-4 days.

Isolation of DNA.

On days 1, 3, 6, and 9 after transfection, the cells were washed twice with buffer solution (PBS), trypsin solution was added, incubated for 10 minutes at 37°C, an equal volume of complete growth medium was added, and centrifuged for 5 minutes at 1.5 thousand rpm. The precipitate was washed twice with PBS and the number of cells in the Goryaev chamber was counted. DNA isolation was performed using the "DNA EXPRESS" kit according to the protocol recommended by the manufacturer. The samples were dissolved in a TE buffer and stored at t -20°C.

Real-time PCR monitoring. Sequencing.

The transfection efficiency for the VEGF plasmid was 10%.

Increased VEGF expression in MMSC culture was observed with an increase on days 6 and 9.

The level of angiogenesis was assessed on the 30th day after surgery on microscopic sections of the myocardium stained with hematoxylin-eosin, based on determining the number of capillaries, the average capillary diameter (d) measured using an eyepiece micrometer, calculating the density (n) (cap/mm2), capillary exchange surface (CES) and capacity capillary bed (CCB) on mm3 of myocardial tissue. The studyofPh2 in the area of open-heart injury was performed by polarographic method using a copper amalgam - cadmium generating pair.

The radioactivity of nuclear and cytoplasmic cell fractions was determined after differential centrifugation of cardiomyocytes destroyed by homogenization on a liquid scintillation counter Beta 2 (counting efficiency of 3H - 56%).

Statistical processing was performed using the Statistica application software (Statistica package). The results were considered reliable at $p<0.05$.

Parameters of the myocardial microcirculatory	Control group Animal group # 1	Administration of MMSCs with VEGF165 Animal group # 2	Significant difference from group # at p<0.05
Number of capillaries n (per 1 mm^2 section)	3661.0±92.0	4020.0±51.0,0	*
Diameter of open capillaries d (microns)	6.50±0.30	7.03±1.20	
Length of functioning capillaries L (mm/ mm^2	2120.0±80.0	3051.7±103.7	*
Capillary exchange surface area S (mm^2/mm^3)	43.30±0.94	67.04±8.51	*
pO_2 (mm Hg)	18.0±4.8	31.1±2.2	1
Radioactivity of uranyl acetic acid-238 per gram tissue (dry weight), kB	0.79±0.06	1.11±0.11	*

Research results and discussion

The results obtained indicate that a single intramyocardial injection of MMSCs transfected with the VEGF-165 gene under simulated ischemia conditions leads to an increase in the total number of capillaries, compared with the control group, by 8.93%, an increase in the diameter of open capillaries by 7.54%, an increase in the length of functioning capillaries by 30.53%, an increase in the capillary exchange surface area by 35.41% and an increase in the partial pressure of oxygen by 42.12%. In the VEGF-165 group, significant neoangiogenesis occurs, but after 1 month, ischemia compensation is insufficient, and less than in the MMSCs transfected with the VEGF-165 gene, which may be due to the formation of a smaller number of arterioles.

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