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Research Article

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Morphological Characteristics of Results of Treatment of Diabetic Angiopathy

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Abstract

The paper presents the results of experimental studies of two types of biomaterials developed in the laboratory of cellular technologies – angiogenesis stimulants from the umbilical cord and bone marrow source. It is shown that both types of biomaterials possess angioinductive properties. At the same time, the mobilization of the organ vascular bed of the tissue bed occurs early; at a later date (up to 30 days), active vascularization of the biomaterial occurs. Statistical analysis shows more pronounced angioinductive processes with the introduction of umbilical cord blood angiogenesis stimulator. The obtained results allowed to recommend this biomaterial for the treatment of obliterating vascular diseases of the lower extremities on the background of diabetes mellitus.

Introduction

Worldwide, chronic obliterating diseases of the arteries of the lower extremities occur on average in 2–3% of the population, and their frequency increases with age, reaching 5–7% in the seventh decade of life [1, 2]. With obliterating thrombangiitis, Raynaud's phenomenon and diabetic angiopathy, the peripheral parts of the vascular system — small caliber arteries and capillaries — are characterized. Reconstructive surgery (direct revascularization) on the arteries of this category of patients is practically impossible. And today in the world over 2.7-4.5 million high amputations are performed annually for diabetic lesions of the lower extremities [3].

To improve blood circulation, methods for stimulating neoangiogense are actively developed by introducing angiogenic growth factors into the body: vascular endothelial growth factor, fibroblast growth factor, platelet-dependent growth factor, and hepatocyte growth factor [4]. But all these growth factors in the body quickly collapse. There is a need for repeated injections, which causes undesirable effects: pronounced vasodilation with the development of hypotension, the occurrence of vascular neoplasms (hemangioma, etc.), increased thrombogenic effect. In this regard, the search continues for new neoangiogenesis factors, the angiogenic properties of which are maximal, and the side effects are minimized [5].

In the laboratory of cellular technologies of the Central Research Laboratory of the Tashkent Medical Academy, a method of targeted and safe cultivation of nucleus-containing umbilical cord blood cells (NCUCBC) with angioinductive properties has been developed. The purpose of the study is to experimentally substantiate the possibility of using an angiogenesis stimulator in the treatment of ischemic lesions of the lower extremities against the background of diabetes mellitus. To study the angioinductive effect of the inject able form of the NCUCBC biomaterial, we carried out experimental studies.

Material and Research Methods

In the experiment, the processes of restructuring of the vessels of the tissue bed and neoangiogenesis in the transplant itself were studied. Studies were conducted on laboratory rats of the Wistar breed of approximately the same age and weight. An allograft prepared in accordance with the technical requirements of 50 mg was diluted in 1.0 ml of saline and injected into the soft tissues of the hind limb of rats (subcutaneously and intramuscularly) at the level of the middle part of the thigh by 0.5 ml. As a control, a biomaterial was used, differing in the source of nucleated cells, namely from the bone marrow. In the control series of experiments, the biomaterial was introduced according to a similar scheme.

The dynamics of structural changes were studied on days 1, 3, 7, 14, 30, 60, 90, and 120 of the experiment. Animals were removed from the experiment by an overdose of ether. For histological studies, a graft was collected with surrounding tissues, followed by fixation in 10% neutral formalin solution. Serial histological sections were stained with hematoxylin and eosin, as well as according to the method of Van-Gieson. The histological specimens were used to calculate the total area of the capillary lumen per unit area (27.8 thousand sq. Mm) both in the biomaterial bed and in the transplant itself.

The results and discussion

In the early stages (1-3 days), a moderate inflammatory reaction is determined in the tissue bed surrounding the graft, consisting in the reduction of damaged vessels, the expansion of the drainage-depositing unit, the preserved microvasculature. The total lumen of the capillaries increases by the end of the first day to 168.7±11.5 μm^2.

For comparison, the total lumen of the capillaries of the subcutaneous adipose tissue of the thigh of an intact animal is 124.8±7.3 μm^2. This figure indicates a moderately pronounced reactive process on the part of the terminal vascular bed to the introduced biomaterial.

On the third day in the experimental series, the total lumen of the capillaries of the bed rises to 236.7±14.8 μm^2, which indicates further mobilization of the vessels of the local blood flow in the tissues of the recipient. Simultaneously, the transendothelial migration of leukocytes occurs. A moderately pronounced polymorphic cell infiltration is observed in the contact zone. Cellular infiltration is determined between the fragments of the dispersed graft. In the composition of the infiltrate undifferentiated connective tissue cells, macrophages. In the zone of contact with the muscle bed are determined migrating muscle cells. On the third day, the first endothelial cords, forming the lumen of the newly formed capillaries, appear in the experimental group along the marginal zones of the graft.

In the control group, identical cell infiltration processes are observed. The morph metric parameters of the capillary bed of the tissue bed of 162.0±7.4 μm^2 in the considered periods (1-3 days) also practically coincide. The presented results confirm the well-known data that on the first day biological processes developing in the environment of allograft are implemented on the basis of common, local and systemic mechanisms [6].

Subsequently (on day 7), signs of an inflammatory reaction in response to an injection of the biomaterial are preserved in the tissue bed of the experimental series of experiments. The terminal vascular bed in the bed of the graft reaches a maximum density: 480.1±3.8 μm^2. The total capillary lumen reaches the highest value and in the control group - 283.3±7.1 μm^2. However, the numerical value of this indicator during the injection of a regenerator stimulator remains significantly lower in comparison with the experimental group.

On the 14th day in the allograft of the experimental and control groups, the processes of angiogenesis are activated. In the same period, the differentiation of microcirculatory links begins. The total clearance of the formed capillaries in the experimental group (202.2±10.6 μm^2) exceeds that of the control group (153.2±5.2 μm^2). In the described period, the reactive phenomena from the vascular network of the graft tissue bed are noticeably reduced, both in the experimental and control series.

The morphological picture significantly changes by the 30th day of the experiment. The inflammatory reaction of the terminal vessels of the bed is replaced by active angiogenesis in the transplant. The total lumen of the newly formed capillaries in the graft in the experimental series reaches 437.6 ± 8.2 μm^2. At the same time in the control series, this indicator is reliably lower and amounts to 243.01±4.7 μm^2. This fact confirms the morph metric parameters. According to literary data, the processes of vascularization of allograft should be accompanied with their active replacement by loose fibrous connective tissue [7].

In our studies between the bundles of fibers of the biomaterial, numerous cells related to the fibroblastic differenon were detected. In the cellular environment, newly synthesized collagen fibers and an amorphous matrix were differentiated. The described processes of proliferation of connective tissue structures even more pain expressed for 60 days. The gradual reduction of the formed vascular bed in the allotransplant, which started from 30 days, continued in all subsequent periods of the experiment [8-19].

So, on the 90th day of the experiment, the total capillary lumen in the allograft decreased in the experiment to 229.9±6.4 μm^2, and in the control - to 164.3±2.9 μm^2. At this time, all fragments of the biomaterial were surrounded by a capillary network. The processes of gradual reduction of the terminal vessels continued in the next 120 days. In the field of allograft, the formed connective tissue regenerate was represented by fibroblasts and extracellular matrix. The capillary bed of the regenerate had a total clearance of 144.9±2.2 μm^2, and in the main group - 175.8±6.8 μm^2.

Thus, the results of experimental studies have allowed concluding that the early vascular reactions in the area of allograft of the main and control groups are of the same type and have a characteristic phasing. The introduction of nucleated cells resulted in early activation of reactive changes. In the first phase (3-7 days), the vascular bed of the tissue bed was mobilized, including non-functioning capillaries into the bloodstream, and the transendothelial migration of blood cells was activated. In the second phase (up to 30 days), induction of angiogenesis intensified with the activation of vascularization of the graft, most pronounced in the NCUCBC biomaterial (experimental series). In the subsequent periods (from 30 to 120 days) there was a gradual reduction of the formed organ vascular bed. These processes of mobilization of the organ vascular bed in the experimental group with the use of NCUCBC were significantly more pronounced compared with the control group (bone marrow source). The data obtained allow us to recommend this biomaterial for clinical use in the treatment of ischemic lesions of the lower extremities.

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