

The Methods of Biotechnology in the Vascular Transplantology

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Abstract

To study the growth factor's influence on the vascular prosthesis and arterial pitch healing we used PTFE ("GORE", "IMPRA"), flourolavsan prostheses (Russia), xenoprostheses and tissue growth factor (GF) we have obtained in our laboratory. The endothelial cells (EC) were cultured under typical condition (1). Bioprostheses endothelized by autocytes were implanted in dogs infrarenal aorta (78). The morphological investigation of functioning endothelized xenoprostheses showed that endothelialization of vascular substitution is a method of stimulating early healing of graft and provides forming of athrombogenal EC monolayer in the first days after implantation. The treatment of prostheses by GF was made by the original method. Experimental animals were divided into 4 groups depending on increasing GF concentration on the prosthesis wall: 0.05 mg/cm² of prosthesis - 0.00025 mg/cm² of prosthesis. There were made operations of grafting the carotids (54), femoral arteries (48), infrarenal part of abdominal aorta (174), torakoabdominal shunting (16). The best results were obtained in the third and the fourth groups. In 4 - 6 weeks after implantation all experimental prostheses and pitches had good cellular organization of neointima and endothelial coverage of luminal surface that was kept for the 6 months of observation.

Keywords: *growth factors, vascular prosthesis, healing, neoangiogenesis, endothelial cells*

Introduction

Critical ischemia of lower limbs determined by arterial systems damage occurs in 50-70% of vascular pathology cases and needs serious reconstructive interferences [2,3,4,8]. The problem of creating adequate vascular substitutions for last ten years practically has not changed. The question of adequate alternative of vascular substitution is as penetrating as it was earlier, especially in grafting of vessels of middle and small diameter [5,6]. Investigation of reasons of vascular grafting failures brings to light main defects of vascular prostheses healing. Obviously, the key principal of qualitative healing of vascular substitute is forming of new vascular wall that has natural biological defense systems. Limited possibilities of growth of endothelium and subendothelial structures on surface of prosthesis, and also absence of valuable

neomedia and neoadventitia forming are not able to provide quantitative functioning of vascular substitute.

For providing the valuable healing of vascular substitute, that has natural biological structures, we used cultivated endothelial cells (EC) and tissue growth factor (GF) obtained from the artiodactyls [7,9].

Material and Methods

The material for the investigation was: prostheses from PTFE of firm "GORE", "IMPRA", USA; flourolavsan prostheses; xenobioprostheses, made by the method of the First Moscow Medical Institute after I. M. Sechenov and firm "Solco" called "Solcograft-P" (Switzerland).

The growth factor was obtained from the brain of artiodactyls. The analysis of albuminous solution of obtained material was made on polyacrylamide gel plates, comprising 10%-ed dividing and 3%-ed concentrating gel in presence of dodecyl sulphate of natrium.

For the endothelial cells receive was used humans umbilical cord veins (550), vena saphena magna (106).

There were investigated the attachment, the growth and covering of human endothelial cells on the intraluminal surface of prostheses by authors methods [1]. The mitogen activity of growth factor (GF) obtained from calf's brain in our laboratory was investigated on the growth of endothelial cells' culture in different concentrations in proportion to heparin 2:1. Were investigated the following concentrations of GF: 100; 200; 400; 600 (g/ml of medium).

Endothelial cells (EC) were obtained and cultivated by described method [1]. The entire prostheses were covered with EC, turning them along their long axle. For this effect mechanism of circumgyrating the prosthesis filled with suspense of cells in horizontal plane was constructed. The instrument allows to change frequency of circumgyration from 0.5 to 1 turn/min.

For earlier forming of endothelial covering of prosthesis' luminal surface we used tissue GF, which we obtained in our laboratory. The treatment of prostheses by the GF was made by the original method. Prostheses were passed through alcohol battery of 30%-50%-90%-100% for 5 min in each concentration. Then they were dried in the air for 2-3 min and placed in heparinized autoplasm. Plasma was taken from the dog being planned for operation 1-2 days before the operation. GF was added in autoplasm in 4 different concentrations: 10.0 mg% - 5.0 mg% - 0.5 mg% - 0.05 mg%. Antibiotics, prednizolon were added to the plasma in quantity that is used for cultures of tissues and cells. The redundant heparin effect was removed by supplement natrii protaminsulfurici after the prostheses had been placed in the prepared plasma. The prostheses were stayed there at 37° C for 2 hours and after that were stayed there at 40° C. Next day the graft was implanted.

Experimental animals were divided into 4 groups depending on increasing mitogen concentration in solution: the first group of animals, 21 experiments, prostheses were treated with the solution of autoplasm, that had 10 mg% GF (0.05 mg/cm² of prosthesis); the second group of animals, 21 experiments, prostheses were treated by the solution of autoplasm that had 5mg% GF (0.025 mg/cm² of prosthesis); the third group of animals, 113 experiments, GF was added to autoplasm in quantity of 0.5 mg %

(0.0025 mg/cm² of prosthesis); the fourth group of animals, 136 experiments, GF was added to autoplasm in quantity of 0.05 mg% (0.00025 mg/cm² of prosthesis).

There were made operations of grafting the carotids (54), femoral arteries (48), infrarenal part of abdominal aorta (174), torakoabdominal shunting (16).

Results

Received GF comprises no less than 13 albuminous components, with molecular mass in measures of 20×10³-70×10³ Da.

Quantity of endothelial cells of culture with the use in absolute growth medium of obtained mitogen in concentration 200µg/ml in 24 hours was (60.3±1.8×10³), increased twice in 48 hours and became 100.2±9.2 ×10³ and in 72 hours 240.2±2.0×10³. Quantity of cells of culture with the use in absolute growth medium of the mitogen in concentration 400µg/ml in 24 hours was 55.4±7.4×10³ and authentically was not different from the quantity of cells in 24 hours with the use of GF in concentration 200µg/ml. The increase of concentration of GF to 600 µg/ml didn't increase the number of cells.

Experimental Examination of Endothelialised Arterial Biotransplants

Frequency of circumgyration of the prosthesis with suspense of EC equals 0.05 turn/min., consistence of the attached cells is closed to monolayer (0,95±0,15×10⁶ cells/cm²).

Endothelialised xenoprostheses (78 experiments) were sewed in abdominal aortas of dogs.

The morphological investigation of functioning of endothelialised xenoprostheses in abdominal aorta of dog shows, that in all cases, beginning from the third day after implantation neointima is formed. The base of its forming is EC artificially seeded on the surface of prosthesis.

In 3-7 days after implantation endothelial monolayer isn't solid. The structure of the prosthesis is seen through disengaged cells. In a week cells solidly cover the surface of the prosthesis. There is no doubt that EC are healed and are able to function on the surface of the prosthesis.

Luminal surface continues transforming in 14 days after implantation. Cells have orientation on blood flow. Endothelial plate is solid, through it isn't seen the connecting fibers. Smooth muscled cells that in attitude

to endothelium have vertical location are situated under the endothelial cover. Thickness of xenoprostheses sprouts with fibroblasts that are orientated along the long axle of the prosthesis.

In 60 days after implantation the inner surface of endothelial xenoprostheses "Solcograft-P" macroscopically doesn't differ from inner surface of aorta. The central part of xenoprosthesis "Solcograft-P" by this time forms a plicated surface with the direction of the plaits by the blood flow. Nucleus prominences are well noted on both the plaits itself and interplated hollows. Surface of the prosthesis is free from impositions of fibrin and cell blood elements.

Experimental Examination of arterial transplants treated with GF in vivo

First and second groups of animals (42 experiments)

The prostheses were treated with solution of autoplasm containing 10 mg% (0.05 mg/cm² of prosthesis) and 5 mg% of GF (0.025 mg/cm² of prosthesis).

In these groups beginning from the first day the prostheses thrombired for the first 2 weeks after implantation. Using prostheses from PTFE and flourolavsan in first days after implantation was noted the forming of neointima with the thickness much superior than the thickness of prosthesis. In this case neointima is parietal thrombus on different levels of organization.

Third group of animals (113 experiments)

GF was added in autoplasm in quantity 0.5 mg% (0.0025 mg/cm² of prosthesis).

Neovascularisation of prostheses from PTFE and flourolavsan on the 7-10 24 hours is noted well formed penetrated with vasa vasorum neoadventitia, snug against surface of the prosthesis. The thickness of prosthesis is penetrated with cells mesenchyme row. Investigation with scanning electro microscopy showed, that in two weeks in position of carotid aorta region of anastomosis is completely covered with solid cell layer, that has identical construction in comparison with the cell layer of aorta. Prostheses in position of carotid and femoral arteries thrombired during first week after implantation.

Fourth group of animals (136 experiments)

Growth factor was added in autoplasm in quantity of 0.05 mg% (0.00025 mg/cm² of prosthesis).

Patency of prostheses is 90 % during 6 weeks after implantation.

In first two weeks anastomoses of prostheses are smooth, shining, color isn't different from the color of intima of grafted vessel in 1.5-2 cm from anastomosis. It isn't noted the narrowing of prosthesis opening in position of carotid and femoral arteries, where diameter of prosthesis is 4mm. interstice of prosthesis are filled with fibroblasts, smoothmusclcd cells that are got over intraluminal surface of prosthesis, where neointima is already formed.

Anastomoses of prostheses are without any sign of hyperplasia. Organized neointima on the place of wedge-shaped thrombus is thin, covered with endothelial cells that increase form the side of artery. Rugosity of surface of artery gets over the surface of prosthesis.

Central part of prostheses has whitish appearance, there are noted solitary punctate impositions of red color on it's background. It is noted celled organization of neointima in the centre of prosthesis. Cells of oblong form are directed to blood flow and compile solid monolayer. Flourolavsan prostheses remained passable during the investigational period -180 days, in 100% of cases. Beginning from the first days after implantation the forming of neoangiogenesis continued similarly. Already in a month, intra luminal surface of prostheses was covered by organized by neointima endothelial monolayer.

Discussion

The key principle of qualitative healing of vascular substitute is forming of new vascular wall that has natural biological defense systems. Limited possibilities of endothelium growth and subendothelial structures on surface of prosthesis, and also absence of forming complete neomedia and neoadventitia aren't able to provide with qualitative functioning of vascular substitute. The use of biological technologies during transplantation of vessels supplies with natural ways of growth of vascular wall structure.

Cultivated endothelium applied on the surface of vascular prosthesis in organism after implantation is powerful regulator of growth of vascular wall. There is no doubt, that the healed endothelial cells are able to function on the surface of prosthesis. If in control prostheses, indispensable condition for forming of endothelial monolayer is the forming on its surface connecting tissue, than in our case endothelium itself forms basal membrane and exerts stimulating influence on the healing of the prosthesis.

Forming of neointima cannot take place isolated and is associated with forming of two other layers: neointima and neoadventitia. Absence of rigid connecting-tissued capsule around prosthesis is prerequisite for forming elastic structures of vascular substitute wall. Obviously, connecting-tissued capsule doesn't form because the prosthesis is already surrounded from one side with endothelium and from the other side loose neoadventitia with big quantity of vasa vasorum and microvascular cell.

Presence of valuable endothelium, as on intraluminal surface, so in periprostheses tissues provides with prosthesis normal functioning from one side and is guarantee of self-regulation of processes of vascular wall regeneration.

In our opinion, consecutive growth of structural elements of arterial wall, stimulated on early levels of healing of prosthesis is able to form functionally valuable new vessel of the circulatory system. In capability of mitogen we exuded GF from brain of calf.

The investigations showed that obtained factor of growth has mitogen activity in respect of endothelial

cells in all investigated concentrations. For cultures of cells with more effective concentration of GF is 200-400 µg/ml in combination with 100-200 µg/ml of heparin. It is necessary to mark that in no cases there was not noted uncontrolled growth of cells.

High concentration of GF during the treating of vessels contributes to precipitate fibrin on the surface of prosthesis and stimulates excessive growth of cells of periprostheses tissues that prevents from evolution of neoangiogenesis processes. Decrease of concentration of GF to 0.00025 mg/cm² of prosthesis wall allows to reach moderate stimulation of growth of EC as from the side of grafted artery, so of microvascular endothelium. Early forming of endothelial pavement is prerequisite for complete healing of prosthesis.

The use of GF precipitates the healing of vascular substitutes, forms neointima covered with monolayer of endothelial cells earlier than there is formed fibrose capsule. Cell organization of neointima becomes possible in absence of rude connecting tissue on intraluminal surface of prosthesis.

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Методы биотехнологии в сосудистой трансплантологии

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Р Е З Ю М Е

Для обеспечения полноценного приживления сосудистого заменителя, были использованы протезы из политетрафторэтилена (ПТФЭ) "GORE", "IMPRA" (США); фторлонлавсановые протезы; биопротезы из сонной артерии крупного рогатого скота 1 ММИ им. И.М. Сеченова и "Solcograft-P" (Швейцария) и тканевой фактор роста (ФР) полученный из мозговой ткани парнокопытных животных. Эндотелиальные клетки культивировали в типичных условиях. Эндотелизированные аутоклетками ксенопротезы были вшиты в брюшную аорту собак (78). Морфологическое исследование функционирующих эндотелизированных ксенопротезов показало, что эндотелизация сосудистых заменителей - это метод, стимулирующий раннее приживление протезов и обеспечивающий формирование атромбогенного эндотелиального монослоя в первые дни после имплантации. Эксперименты с использованием ФР были разделены на 4 группы в зависимости от его содержания в растворе: 0.05 мг/см² протеза - 0.00025 мг/см² протеза. Произведены операции: протезирование сонных артерий (54), бедренных артерий (48), протезирование инфраренальной части брюшной аорты (174), торакоабдоминальное шунтирование (16). Наилучшие результаты получены в третьей и четвертой группе животных. Через 4-6 недель после имплантации все экспериментальные протезы имели хорошую клеточную организацию неointимы и эндотелиальное покрытие люминальной поверхности сохранялось на протяжении 6 месяцев наблюдения.

Ключевые слова: фактор роста, протезирование сосудов, заживание, неоангиогенез, эндотелиальные клетки