USE OF HUMAN FETAL LIVER CELLS FOR TREATMENT OF PATIENTS WITH LOWER LIMB PERIPHERAL ARTERY DISEASE

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ABSTRACT

In the group of patients (n = 21, mean age 54 ± 5.8 years) with chronic lower limb ischemia stage IIB who were non-liable for reconstructive-restoration surgery, we have established positive clinical effects of local transplantation of human fetal liver progenitor cells. Complex examination following 1, 3, 6 and 12 months after transplantation included duplex scanning of limb arteries, x-ray contrast arteriography and laser Doppler flowmetry as well as measuring pain-free walking and evaluating life quality based on individual questionnaire data. Owing to the transplant “Cryopreserved human fetal liver progenitor cells” the patients demonstrated stable increase of life quality index and pain-free walking as well as improvement of general health allowing assign them to the group of patients with lower ischemia stage, quicker social rehabilitation and lesser risk of disabling surgery (p < 0.05). Also, there were observations of improved microcirculation in the ischemic extremities owing to activation of endothelium-independent mechanisms of vasodilatation, reduced myotonus and neurotonus of the pre-capillaries and improved endothelium-dependent influence on the microhaemodynamic and, hence, an increased reserve capillary blood flow (p < 0.05). Analysis of the obtained results indicates prospects and effectiveness of using fetal liver cells transplantation in the patients who are not liable for surgical reconstruction of the vascular bed.

KEYWORDS: chronic lower extremity ischemia, stem cells, fetal liver, cell transplantation.

MATERIALS AND METHODS

The investigation was carried out by the staff of the A. A. Shalimov National Institute of Surgery and Transplantology of the National Academy of Medical Sciences of Ukraine in accordance with the order of Health...
The main clinical group consisted of 21 patients (mean age 57.4 ± 5.8) with IIIb-IV degree chronic lower limb ischemia. In terms of their anatomic-functional condition, all of them were found fit for reconstructive-restoration intervention and were judged to have no risk for post-operation thrombosis or extremity amputation. According to the clinical forms the patients were divided in the following way: obliterating endarteritis (n = 4) and obliterating atherosclerosis (n = 17). 17% of the patients had trophic disorders like local necrosis or soft tissue defects of the feet and toes.

Part of the patients (45.4%) was operated earlier. To reduce ischemic lesions, we used the methods of direct and indirect revascularization (Tab. 1). Besides, all of the patients had earlier received conservative treatment (prostaglandin E preparations and ilomedin).

The patients of the main group underwent transplantation of cell transplant (“Cryopreserved fetal liver progenitor cells). The average dose for transplantation was 56.8 ± 6.2•10⁶ nucleated cells per patient. Transplantation was performed in ambulatory conditions under local anesthesia via single local injection of cell transplant: subfascially, into ischemic muscle of the leg and/or hip, along obliterated vessels (Fig. 1). Main cell injection zones:

1. The leg – three injection loci on the medial side and two loci on the lateral side;

2. The foot – between toes and from medial side under foot aponeurosis;

3. The muscles, if need be, through 2 access points on the medial side. Cell transplant, product of the Institute of Cell Therapy (Kyiv, Ukraine), was the suspension of progenitor cells isolated from human liver embryos of 6-12 weeks gestation and stored in liquid nitrogen at -196 °C. Cell transplants were obtained from abortive embryos received during voluntary disruption of pregnancy on the informed consent of women. The obligatory requirement for obtaining biological material was the absence in women of any autoimmune and systemic diseases, viral hepatitis, degenerative neurological pathology, bacterial infection, septicemia, syphilis (in anamnesis or treated), and icterus of unknown etiology. Oncologic diseases in the anamnesis of pregnant women, use of radiotherapy of chemotherapy, intake of hormonal drugs, toxic or narcotic substances were also contraindications for receiving biological material. Women with intrauterine growth restriction and HIV risk group also excluded. In addition, donors of biological material tested for the TORCH infections, syphilis, HIV, hepatitis B and C, mycoplasma.

Before using, the cryovials with transplant were unfrozen on water bath at 38-40 °C. Then 1.5 ml of cell transplant was taken into syringe and diluted to 10 ml by isotonic NaCl solution.

The indications for cell transplantation were as follows: absence of any possibilities for direct revascularization, namely: occlusion of distal (terminal) arterial segment and/or presence of multifocal lesions of arterial vascular bed combined with «unreconstructable» injury of peripheral segment; chronic ischemia of the extremities IIIb-III stage; chronic ischemia of the extremities IV stage without acute pyoinflammatory processes and fungal infection of the feet; absence of oncopathology in the anamnesis and negative test on oncomarkers.

The contraindications for cell transplantation were the followings: possibilities for «direct» revascularization, mycotic lesions of the feet, acute pyoinflammatory processes of the feet, cytomegalovirus infection, and excess oncomarkers and positive oncologic anamnesis.

The patients of the control group (n = 19), who were matched by age and extremity ischemia stage with the main group patients, received conservative therapy with prostaglandin E preparations in the doses according to the existing recommendations [11].

At admission to hospital all patients underwent complex diagnostic examination that included extremity arterial duplex scanning, X-ray contrast arteriography and laser Doppler flowmetry allowing perform non-invasive microcirculation control in real time.

Further the patients’ health was checked at 1-3-6-12 month intervals following transplantation. At each stage of the study, we evaluated microcirculation by laser Doppler flowmetry, made duplex scanning and X-ray contrast arteriography of the peripheral arterial vessels and determined segmental pressure in the affected limb vessels.

In addition, the patients completed personal cards to evaluate life quality and pain-free walking distance and speed by means of the questionnaire W. O. Spitzer, Walking Impairment Questionnaire (recommended by the TransAtlantic Inter-Society Consensus on Management of Peripheral Arterial Disease) [12].

The obtained data were statistically estimated by using Microsoft Office Excel (built 11.5612.5703) and programs for statistical analysis Statgraphics Plus 5.1 Enterprise edition ("Statistical Graphics corp. 2001).
the Fontaine-Pokrovsky scale following cell transplantation was observed in 88.2% of the patients after 1-3 months. The results of the questionnaire evidenced for better life quality and greater pain-free walking distance and walking speed.

However our later observations beginning from 3rd and especially during 6-12th months after treatment disclosed drastic differences between objective and subjective indices in both groups, being in favor of cell transplantation receivers.

According to the questionnaire the results in main versus control group were 2.5-fold better (p ≤ 0.01) by the 3rd monitoring month. At the same time the results of duplex scanning and segmental pressure did not actually differ from the baseline values obtained before treatment in both groups. However the monitoring of microcirculatory bed in main group patients correlated with the results of the questionnaire. The data of laser Doppler flowmetry showed statistical (p < 0.05) decrease of the microcirculation values on basic level from left forearm. Notably, the capillary blood flow reserve increased significantly. If at the beginning of our investigation it made on the average 103.0 ± 45.0 %, after one month it was 229.0 ± 39.0 % (p < 0.05).

There was a tendency towards an increase of the microcirculation indices at the expense of the widening of the existing capillaries and, perhaps, an involvement of non-functioning capillaries into the bloodstream owing to activation of the formation of the new capillary bed. Also, the influence of transplanted cells on the endothelium-independent mechanism of vasodilatation is believed to take place, mainly by reducing myotonus and neotonus of the precapillaries. At the same time the state of the microcirculatory bed of control group patients does not differ significantly from the state that was registered before treatment.

Later, 6 months after the beginning of treatment we could see further improvement of general health condition in the main group patients (1.7-fold increase of questionnaire parameter values) in the comparison with analogous values of 3rd monitoring month (p < 0.05). We also registered the increase of the pain-free walking distance compared with control values and stabilization of ischemia degree according to the Fontaine-Pokrovsky scale.

As the analysis of the obtained results has shown, three months after cell transplantation the four patients with ischemia degree IV passed the Fontaine-Pokrovsky scale. The dynamics of ischemia degree after cell transplantation.

Table 2. The dynamics of ischemia degree after cell transplantation.

<table>
<thead>
<tr>
<th>ISCHEMIA DEGREE (ACCORDING TO FONTAINE-POKROVKY)</th>
<th>THE NUMBER OF PATIENTS BEFORE CELL TRANSPLANTATION</th>
<th>THE NUMBER OF PATIENTS ON THE 6TH MONTH AFTER TRANSPLANTATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>II A</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>II B</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>IV</td>
<td>7</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>19</td>
</tr>
</tbody>
</table>

Besides, one patient with peripheral form of injury underwent leg amputation 4 months after cell transplantation. Against the background of complete safety and passing to ischemia degrees III and IIA an acute arterial thrombosis was diagnosed in the arteria poplitea involving the Hunter’s canal. Surgery was thought ineffective and conservative therapy had a short-term effect. With the worsening of patient’s condition and a development of marked pain syndrome and necrotic processes, the leg was amputated.

The analysis of segmental pressure during entire period of observation indicated the stability of the ankle-brachial index which remained in fact at the pre-transplantation level. That is in our case the given method failed either to give an objective picture or to allow trace changes in the regional hemodynamic.

The duplex scanning showed development of collateral network in transplantation areas formed by medium-size vessels. We noticed a stable tendency to further normalization of the microcirculatory bed owing to improved venous outflow, endothelium-dependent vasodilatation via activation of active and passive mechanisms of regulation of capillary blood flow and increased capillary blood flow reserves (p < 0.05) compared with those of 3rd month of our investigation. That is a stable positive level of capillary blood flow had been formed.

At the same time the condition of control group patients worsened and in fact approached to baseline level. The pain-free walking distance and speed reduced, resting pains appeared, etc. According to the Fontaine-Pokrovsky scale, the degree of ischemia increased. Regress of clinical finding correlated with the results of instrumental study. The segmental pressure indices were reduced and the results of laser Doppler flowmetry evidenced for considerable worsening of the microcirculatory bed state. In other word the control group patients returned to baseline level already at 6th month after conservative therapy and needed a repeated course of such therapy. They actually discontinued their participation in the study.

On the 12th month post-transplantation we noticed fixation of clinical status a so-called «plato» phase that was manifest in the stability of the interviewee’s pain-free walking distance and speed indices. In addition the microcirculatory bed state was stabilized as seen from capillary blood flow reserve increase (326.2 ± 19.2 % (p < 0.01) compared to the baseline levels, myotonus and neotonus of the capillaries.

The microcirculation indicator in the first foot toe did not differ from the control one. Its increase was linked with capillary network expansion and possible formation of newly-formed capillary network, involvement of non-functioning «sleepy» capillaries into microcirculation process and impact of transplanted cells onto endothelium-independent mechanism of vasodilatation mainly owing to reduced neotonus of the pre-capillaries.

Repeated arteriography demonstrated increase of the collateral vascular network in the form of big- and medium-caliber vessels localized along obliterated major arteries and forming anastomoses with their fragments with residual blood filling.

Thus the results of clinical investigation have shown that transplantation of fetal liver progenitor cells (cell transplant «Cryopreserved fetal liver progenitor cells») to the patients with chronic limb ischemia is the effective method of indirect revascularization. As the evidence for clinical efficacy of cell transplantation has been the improved clinical status of patients and activation of microcirculation in the ischemic extremity.
CONCLUSIONS

THE USE OF CELL TRANSPLANT «CRYOPRESERVED FETAL LIVER PROGENITOR CELLS» IN THE TREATMENT OF PATIENTS WITH CHRONIC EXTREMITY ISCHEMIA PROMOTED:

• STABLE INCREASE OF LIFE QUALITY INDEX, PAIN-FREE WALKING, IMPROVED GENERAL HEALTH CONDITION, TRANSFER OF PATIENTS TO LOWER ISCHEMIC STAGE, SOONER SOCIAL REHABILITATION AND LESSER RISK OF DISABLING SURGERY;

• IMPROVEMENT OF THE MICROCIRCULATION OWING TO ACTIVATION OF ENDOTHELIUM-INDEPENDENT MECHANISMS OF VASODILATATION, REDUCTION OF THE MYOTONUS AND NEUROTonus OF THE PRECAPILLARIES, IMPROVEMENT OF ENDOTHELIUM-DEPENDENT IMPACT ON MICROHEOMODYNAMICS AND, HENCE, INCREASE OF RESERVE CAPILLARY BLOOD FLOW;

• SERVES AN EFFECTIVE AND ACCESSIBLE METHOD FOR DIRECT REVASCULARIZATION INDICATED TO THE PATIENTS WITH CHRONIC EXTREMITY ISCHEMIA IN WHOM VASCULAR BED RECONSTRUCTION PROVES IMPOSSIBLE AND IN ELDERLY PATIENTS WITH PRONOUNCED CONCOMITANT PATHOLOGY.

WE ASCRIBE THE POSITIVE CLINICAL EFFECT OF CELL IMPLANTS TO ACTIVATION OF ANGIOGENESIS UNDER INFLUENCE OF INJECTED STEM CELLS LEADING TO THE FORMATION OF NEOCAPILLARY VASCULAR NETWORK ALLOWING BLOOD DELIVERY TO ISCHEMIC LOWER EXTREMITY AREA.

REFERENCES