

CHARACTERISTICS OF LEG MUSCLE TISSUE MORPHOLOGICAL CHANGES AFTER PRP-THERAPY

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The number of people suffering from chronic lower limb ischemia is growing every year. One of the promising treatment directions of these patients is a cell therapy to stimulate angiogenesis. In the study, PRP therapy procedure was carried out using a system of centrifugation "Harvest Technologies, Smart PReP 2", USA. As a result, the 6-month follow-up after PRP-therapy in patients with femoral-popliteal-tibial occlusion is an increase by 58.6% capillaries per muscle fiber and increase the size of the muscle fibers by 61.1%. PRP - therapy is effective and safe method of treating patients with CLI.

Key words: *cell therapy, femoral-popliteal-tibial occlusion*

As the results of international trials, chronic ischemia of the lower limbs were recorded in 5-8 % of patients older than 50 years, and in the presence of risk factors (smoking, diabetes , hyperlipidemia , hypertension) - approximately 30% [1, 2, 3] . Despite the large arsenal of surgical and endovascular interventions, approximately for 20-40 % patients with CLI "direct" methods of revascularization can not be applied due to the anatomical features of the lesion of the great arteries, disease duration and comorbidity [5, 6]. The number of "large" lower limb amputations due to progressive CLI in the European Union and the United States more than 100 thousand per year [4, 5]. One of the promising directions in the treatment of this patient group is cell PRP - therapy for promoting angiogenesis [7] (autologous platelet-rich plasma), which contains a large number of growth factors and biologically active proteins [8,9]. Especially the use of PRP-therapy in vascular surgery remain poorly understood and require further improvement.

The study aim - explore the morphological changes of muscle tissue in patients with "inoperable" types of femoro-popliteal-tibial occlusions after PRP-therapy.

Materials and methods.

The study analyzed the complex treatment of 19 patients with "inoperable" types of femoro-popliteal-tibial occlusions. The average age of the patients was $65 \pm 4,9$ years. Depending on the degree of chronic lower limb ischemia (Fontaine- Pokrovsky classification) patients were divided as follows: IIBst. ischemia verified in 5 patients, IIIst. ischemia verified in 6 patients and IVst. - 8 patients.

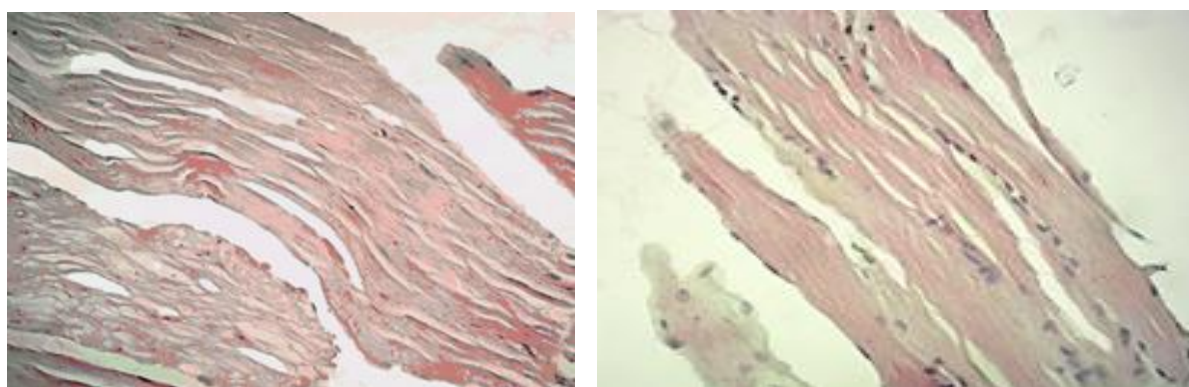
To determine the effectiveness of PRP- therapy as a stimulator of angiogenesis process histological biopsies to obtained shin muscle tissue of patients were conducted. For histological examination, collected the shin muscles ischemic puncture needle «Spring Cut № 16." Puncture was performed before the first session PRP- therapy and repeated procedure was performed after 6 months. Biopsies were fixed in 10 % formalin solution and embedded in paraffin. Serial paraffin sections 5-7 micrometers in thickness. Stained with hematoxylin and eosin. In sections in Review microscopy studied state of muscle fibers, the presence of these nuclei, striations sarcoplasmic and features of the distribution of connective tissue. From eyepiece microscope transverse sections of muscle fibers measured the diameter in micrometers (microns) and the number of capillaries that supply blood to the muscle fibers.

PRP- therapy procedure was performed using centrifugation system "Harvest Technologies, SmartPReP 2", USA. APC +60 set used for autologous platelet-rich plasma and growth factors (Autologous Platelet Concentrate +). PRP- obtained concentrate was administered by intramuscular injection of 20 downstream of occluded arteries in ischemic muscle and shin area trophic changes. From the 2nd day of treatment, all patients performed designed complex exercise aimed to strengthening the muscles of the leg and thigh. Stimulation of angiogenesis in all patients was regarded as the only possible method of improving blood circulation in the affected limb. The results obtained are presented as mean (M) and standard error of the mean (m). Considered statistically significant differences between the indices rejecting the null hypothesis and a significance level of $p < 0,05$.

Results and discussion. As a result of comprehensive treatment in 17 patients managed to keep the lower limb. During the entire period of the dynamic observation it was not recorded, no cases of anaphylactic reaction, necrotic defects or purulent - inflammatory reactions at the injection site PRP- concentrate. In 4 patients with baseline 3 stage of CLI, 6 months after treatment achieved the elimination of pain and pain-free walking distance increased to 200m.

In five patients with IVst. ischemia were also marked positive outcome. Trophic changes decreased, partially healed, the pain threshold fell to 0.

In triceps tibia biopsies taken from patients with CLI before the PRP - therapy, there were ischemic changes of skeletal muscles. Most muscle fibers were of small diameter of $39,3 \pm 1,4$ mm. Their hyperchromatic nuclei, of elongated shape and disposed along the periphery of the muscle fiber. In most of them nucleoli were not found. As part of the muscle cell sarcoplasmic no striation. In preparations found a small amount of single hypertrophied muscle fibers. Hypertrophied myocytes diameter greater than 80 microns. Endomysial connective tissue loosened. Spasmatic arterioles and their lumen narrowed, thickened walls of blood vessels (Fig. 1, A). Almost all patients in biopsies taken away from triceps tibia revealed an increase in connective tissue. Most capillaries without clearance. Number of capillaries per muscle fiber is $3,58 \pm 0,15$.



A.

B.

Fig.1. Skeletal muscle condition before (A) and after the PRP-treatment (B) of the patient 67. With CLI 4 st. Schick reaction with hematoxylin staining of nuclei. Ob.40; col 10.

Within six months after PRP- therapy and regular exercise, all patients reported improved quality of life. With the consent of the patients underwent repeated biopsy of skeletal tissue triceps tibia during the session re- PRP- therapy. Histological examination of biopsy specimens showed a significant improvement in the morphological picture of the skeletal tissue triceps tibia (Fig. 1,B). In preparations simplast all patients revealed a sharp increase in the transverse dimensions of the muscle fibers. Preferably they have a diameter of $63,3 \pm 1,4$ mm ($p < 0,05$). They increased the number of nuclei (Fig. 1, B). Sarcoplasm of muscle cells stained more intensely when setting CHIC - reaction. On longitudinal sections of muscle fibers

clearly visualized sarcoplasmic transverse striations. Clearly manifested capillaries, which mainly clearance disclosed . The number of capillaries per muscle fiber was significantly increased to $5,6 \pm 0.2$. In preparations of all patients revealed a sharp increase in the number of hypertrophied muscle fibers. Preferably they have a diameter greater than 80 microns. In the connective tissue lymphocytes are detected. Simultaneously observed increase in endomysial connective tissue. Thus, six months after the PRP- therapy, in preparations of biopsy specimens in all patients significantly increases the number of capillaries per muscle fiber, which is the basis for improving the blood supply to the muscle tissue in which the phenomena of muscle fiber hypertrophy are observed.

Conclusion:

1. Application of PRP-therapy in patients with occlusion of the femoral-popliteal-tibial segment increases the number of capillaries by 58.6% per muscle fiber.
2. Six months after the PRP-therapy and regular exercise the transverse size of the muscle fibers of triceps tibia increased by 61.1%.
3. PRP-therapy is effective and safe method of treatment for patients with occlusion of the femoral-popliteal-tibial segment.

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ХАРАКТЕРИСТИКА МОРФОЛОГИЧЕСКИХ ИЗМЕНЕНИЙ МЫШЕЧНОЙ ТКАНИ ГОЛЕНИ ПОСЛЕ PRP-ТЕРАПИИ

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Количество людей, страдающих хронической ишемией нижних конечностей, с каждым годом растет. Одним из перспективных направлений в лечении пациентов с этой патологией является клеточная терапия для стимуляции ангиогенеза. В ходе исследования всем больным проводили процедуру Platelet Rich Plasma (PRP), используя систему центрифугирования "Harvest Technologies, SmartPReP 2", США. Применение PRP-терапии у пациентов с окклюзией бедренно-подколенно-берцового сегмента артериального русла приводит к увеличению числа капилляров на одно мышечное волокно на 58,6% и увеличению размера мышечных волокон на 61,1%. PRP-терапия – эффективный и безопасный метод лечения пациентов с хронической ишемией нижних конечностей (ХИНК).

Ключевые слова: клеточная терапия, бедренно-подколенно-берцовые окклюзии

ХАРАКТЕРИСТИКА МОРФОЛОГІЧНИХ ЗМІН М'ЯЗОВОЇ ТКАНИНИ ГОМІЛКИ ПІСЛЯ PRP-ТЕРАПІЇ

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Кількість людей, що страждають на хронічну ішемію нижніх кінцівок (ХІНК), з кожним роком зростає. Одним з перспективних напрямів у лікуванні пацієнтів цієї групи є клітинна терапія для стимуляції ангіогенезу. В ході дослідження всім хворим проводили процедуру PRP-терапії, використовуючи систему центрифугування "Harvest Technologies, SmartPReP 2", США. Застосування PRP-терапії у пацієнтів з оклюзією стегново-підколінно-гомількового сегмента призводить до зростання числа капілярів на 58,6% на одне м'язове волокно та збільшення розміру м'язових волокон на 61,1%. PRP-терапія – ефективний і безпечний метод лікування пацієнтів із ХІНК.

Ключові слова: клітинна терапія, стегново-підколінно-гомількові оклюзії