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Трансплантація аутологічних стовбурових клітин, виділених із жирової тканини, в комплексному лікуванні хронічної ішемії нижніх кінцівок

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Ttansplantation of autologous stem cells, obtained from adipose tissue, in complex treatment of chronic ischemia of the lower extremities

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Реферат

Мета. Дослідити ефективність використання аутологічних стовбурових клітин у лікуванні хворих з критичною хронічною ішемією нижніх кінцівок (ХІНК).

Матеріали і методи. З 2012 по 2015 р. проведено лікування 41 хворого з ХІНК. Чоловіків було 22 (53,7%), жінок – 19 (46,3%). У 1–й групі (основній) 21 (51,2%) пацієнт отримував стандартний курс лікування з використанням аутологічних мезенхімальних стовбурових клітин. У 2–й групі (контрольній) 20 (49,8%) пацієнтів отримували стандартний курс консервативного лікування простагландинами групи Е1 (ПГЕ1). Результати досліджували, вимірюючи кісточково–плечовий індекс, показники лазерної дуплексної флоуметрії та дистації ходьби.

Результати. Впродовж однорічного спостереження зафіксували достовірне збільшення показника мікроциркуляції у хворих, яких лікували, використовуючи аутологічні мезенхімальні стовбурові клітини, в порівнянні з хворими, які отримали курс терапії ПГЕ1 (p=0,037). Позитивну динаміку показника мікроциркуляції у разі використання ПГЕ1 спостерігали протягом (1,8 ± 0,3) міс, а у разі використання аутологічних мезенхімальних стовбурових клітин показник мікроциркуляції достовірно збільшувався через (2,7 ± 0,4) міс та залишався таким упродовж однорічного спостереження.

Висновки. Розвиток терапевтичного ефекту у групах пацієнтів, яких лікували аутологічними мезенхімальними стовбуровими клітинами та ПГЕ1, не збігався за часом, отже, раціональним можна вважати комплексне лікування з одночасним використанням обох методів та проведенням дозованого фізичного тренування.

Ключові слова: мезенхімальні стовбурові клітини; критична хронічна ішемія нижніх кінцівок; лазерна дуплексна флоуметрія; електрозварювання живих тканин.

Abstract

Objective. To investigate the efficacy of application of autologous mesenchymal stem cells (AMSC) in treatment of patients with chronic critical lower limb ischemia (CCLLI).

Materials and methods. In 2012 – 2015 yrs there was conducted the treatment of 41 patients, with CCLLI: 22 (53.7%) – men, and 19 (46.3%) – women. In the Group I (the main) 21 (51.2%) patients have had obtained a standard course of treatment, using AMSC. In the Group II (the control one) 20 (49.8%) patients have obtained a standard course of conservative treatment, using prostaglandins of group E1 (PgE1). The results were investigated, measuring ankle–brachial pressure index, and the indices of a laser duplex flowmetry and a walking distance.

Results. During a one–year follow–up a trustworthy enhancement of microcirculation index was registered in the patients, who were treated, using AMSC, comparing with the patients, who obtained a course of therapy of PgE1 (p=0.037). Positive dynamics of the microcirculation index while a PgE1application was observed during (1.8 ± 0.3) mo, and while application of AMSC the microcirculation index have been enhanced trustworthily in (2.7 ± 0.4) mo and persisted during one–year of follow–up.

Conclusion. Development of therapeutic effect in groups of patients, who were treated with AMSC and PGE1, did not occur simultaneously, thus , a complex treatment with simultaneous application of both methods and conduction of a dosed physical training may be considered a rational option.

Keywords: mesenchymal stem cells; critical chronic ischemia of the lower extremities; laser duplex flowmetry; electro–welding of living tissues.

Introduction

Peripheral artery disease (PAD) is a significant medical and social challenge for both patients from low–income and developed countries. According to recent statistical reports, more than 250 million people all over the world are suffering from PAD, the majority of whom represent the developing countries. The global PAD prevalence has increased by 24% during 2000–2010 from 164 million to 202 million cases. Approxi-

mately 70% of all patients, namely 140.8 million people, live in regions with low or middle income per capita, with PAD incidence increased among them by 29% over the past decade. However, even high–income regions show an increase in PAD incidence by 13% over the same period of time. [1,2].

One of the advanced PAD stages such as chronic critical lower limb ischemia (CCLLI) still remains an important medical and economic problem since it leads to early disability and increases the mortality rate for working–age people. The high prevalence of the disease and ineffective treatments (surgical interventions aimed to restore the patency of arterial vessels and make bypass shunts) cause the need for further research and development of fundamentally new approaches to PAD therapy [3]

Therapeutic approaches based on regenerative potential of stem cells have been recently becoming more common in treatment of a wide range of autoimmune, cardiovascular, respiratory, renal diseases as well as diabetes mellitus and diseases of the musculoskeletal. The long–term results of CCLLI treatment by stem cells and its modified use are a promising prospect in the fight against lower limb vascular pathology [4–8].

Experimental treatment model by adipose tissue derived stem cell cultures for chronic critical limb ischemia in rabbits. The research team has previously carried out a pilot study on animals to investigate the morpho functional state of the rabbit lower limb under the condition of CCLLI model and application of syngeneic stem cell cultures derived from the rabbit adipose tissue (ADSCs). For this reason, 18 rabbits (female) were selected and then divided into 3 groups: pseudo–operated (I), operated with 0.9% NaCl solution administration but without ADSCs introduction (II), with ADSCs introduction (III). The CCLLI was modelled in animals utilizing the technology of electric welding of living tissue (femoral artery ablation was performed by appropriate tools). The electric welding technology was designed in Ukraine with several advantages over commonly known approaches, namely relatively low temperatures at the sites of application. The morpho functional state of the lower limbs was assessed by duplex flowmetry, functional tests and pathomorphological study. As a result, a partial restoration of pelvic limb function and histological improvement of muscle tissue were found in Group III. The Group II did not show any function recovery or histological improvements. The partial epithelization of trophic ulcers may have suggested lower rates of necrotic processes in the Group III. The study concluded that the use of ADSCs, in terms of CCLLI modelling in rabbits, affected the restoration of the lower limb function, which was tested by qualitative and quantitative changes in morphological and functional parameters 5 weeks after the cell introduction.

Material and methods

During the period from 2012 to 2015, 41 patients with CCLLI were included in the study: 22 (53.7%) males and 19 (46.3%) females. The patient average age in the main group was equal to 64.2 ± 4.1 years, control 65.1 ± 3.9 years. The symptoms lasted from 3 weeks to 2 years.

The study included patients with non–reconstructive occlusive and obliterating diseases of the lower limb arterial vessels with confirmed CCLLI stage II–IV according to the Fontaine classification.

Patient exclusion criteria:

– Clinically significant laboratory test results beyond the normal range.

– Surgical or other invasive intervention of the affected limb during

the last 3 months before the treatment.

– Myocardial infarction during the last 6 months before the treatment.

– Any kind of patients mental or psychological state, which could enable to understand the written and spoken instructions, in particular related to information covering the risk or inconvenience due to participation in the study.

– Reconstructable diseases of the lower limb arteries.

– Septic gangrene found in patient.

– Heart failure (stage II and IV according to NYHA).

– Malignant neoplasms.

All patients were divided into two groups. The first group (the main group) included 21 patients (51.2%) that received a standard course of treatment with autologous mesenchymal stem cells (AMSC). The second group (the control group) included 20 (49.8%) patients that underwent standard course of conservative treatment with prostaglandin E1 (PgE1). At the same time, both groups received a standard course of therapy which included: low molecular weight heparins, statins, solutions for improving blood rheological properties. With respect to the age distribution, CCLLI stages, incidence of adjacent pathology, no significant difference was found between the main and control groups.

The control group patients received conservative therapy with prostaglandins E1 (alprostadil, "Vazoprostan"). Alprostadil was administered intravenously once a day using a venflon type catheter: 60 μg of alprostadil were diluted with 200 ml of a physiological saline administered as a 2–hour infusion. A single course of treatment lasted for 30 days. Along with introduction of alprostadil therapy, patients followed dosed physical exercises according to the procedure described below.

The microcirculation rate was determined in both patient groups by laser doppler flowmetry, the ankle–brachial pressure index (ABPI) and treadmill test. Studies were carried out after hemodynamic stabilization and patient acclimatization to the room conditions at a temperature of 22–23 °C according to the methods described below. All the patients were re–examined on the last day of the therapy course, 30 days, 90 days and 1 year after the treatment start.

The autologous mesenchymal stem cell therapy was given to 21 patients. Based on a number of studies and clinical analysis, cell–based therapeutics could be administered via two common ways or its combination. The first technique involves a cell introduction directly into the ischemic muscle along the obliterated vessels. In such technique the administered cells immediately come into a contact with ischemic mediators which stimulate neo–angiogenesis and could lead to blood vessel formation. The second technique exploits both intravenous and intramuscular routes of administration to increase the therapeutic effect of AMSC.

The nature of a medical intervention in the study was divided into two stages. The first stage aimed to collect the cell

material and transfer it to a specialized laboratory for the stem cell cultivation. The second stage intended to administrate the AMSC to a patient.

The cellular material was collected by lipoaspiration from the subcutaneous tissue of the abdominal region or a single skin flap removal. The technique was performed under sterile conditions and local anaesthesia of the patient.

The obtained biomaterial was transferred to the laboratory under sterile conditions for the following stem cell cultivation.

Three doses of cellular material were cultivated in the range of 12–15 days. The average dose of stem cells in the resulting material was 10–13 × 10^6 cells. Afterwards, the material was sealed in a sterile plastic tube with a 15 ml volume. The contents of the test tube included living patient's mesenchymal stem cells and the physiological saline. The liquid phase did not contain any preservatives and other auxiliary substances. The material was transported together with a quality certificate. We have recalculated the number of cells in the material that appeared to correspond with the quality certificate.

During the second stage, the cultured AMSC were administered to the patient three times with a 2–3–week interval according to the standard scheme. At this stage manipulations were performed without anaesthesia that did not cause any unpleasant sensations or discomfort and were well tolerated by patients. The first cell administration was carried out by an intramuscular injection (0.3–0.5 million cells) in the shin of the affected limb. The second cell administration combined both intramuscular and intravenous routes. 5 ml of cell suspension (~2 million cells) was slowly introduced via the intravenous injection previously diluting this dose at 34–36 °C in the physiological saline. The third cell administration was performed using the method of intravenous injections alone. The total amount of cultured cells administered to the patient for the entire course was equal to 31–38 × 10^6 cells. The stem cell administration was accompanied by patient's physical exercises according to the procedure described below.

Results

No significant differences were found between the control and main groups. According to the plan, the following check–ups were carried out on the last day of the treatment and 1 year after the treatment start.

Laser doppler flowmetry study results. Laser doppler flowmetry (LDF) provides an integral assessment of skin capillary blood flow. Data measured before the treatment start indicates a decrease in the LDF–signal as the arterial ischemia progresses as well as a change in 100% of cases in its rhythmic structure. There were no statistically significant differences between the control and main groups based on LDF parameters before the treatment (p = 0.854), the two patient groups were comparable in terms of microcirculation.

The study has also found a violation of low (characterizes the active microcirculation mechanism) and high (characterizes an increase of parasympathetic effects) frequency rhythm relationship in patients with CCLLI. In such case so–called "spectral constriction" was observed in LDF–grams. Under normal conditions, the amplitude of vasomotions (ALF) is equal to 20–25% of the LDF signal level, whereas under CCLLI conditions ALF decreases down to 6 – 10% at a frequency of 6 – 8 oscillations per minute. The power of the blood flow LF–oscillation spectrum was evaluated by its contribution to the general spectrum of flaxomotions that was progressively decreasing as the severity of the disease was increasing. However, the most significant changes were observed in the range of HF– and CF–oscillations. Thus, the contribution of HF–oscillations to the general spectrum in patients with CCLLI has increased up to 15%, while in the normal range is about 5%; in the cardio rhythm range it also increases up to 6 – 7% (under normal conditions 1,1 – 1,3%). The relationship between the degree of severity caused by changes in flaxomotions) amplitude and CCLLI stage defined by the degree of arterial circulation damage was concluded to be in a direct relationship.

The obtained results suggest a close correlation between the systolic segmental pressure, ABPI and LDF–gram indexes, macro– and microhemodynamics indices which supports the data from a series of other studies. In the same way, the flaxomotions characteristics of the skin blood flow can be used to differentiate CCLLI stages, especially in case of critical and severe stages. The research confirms the following conclusions, since normalization of LDF–grams was observed after revascularization of the ischaemic limb.

Table 1 shows the results obtained from the preliminary analysis of AMSC and PgE1 therapy on the last day of the treatment. No significant differences were found between the main group and subgroups (p = 0.56). The results also demonstrate an improvement in LDF results among the control group patients that evidences the early therapeutic effect of PgE1.

Tables 2–4 provide the experimental data of LDF in patients following 30 days, 90 days and 1 year after AMSC or PgE1 treatment course.

The control group patients showed the therapeutic effect of alprostadil immediately 15–25 days after the treatment. As a consequence, a slight increase in the microcirculation was found for the following 1.6 to 2.6 months. The therapeutic effect caused by PgE1 use had a tendency to reduce and so demonstrating microcirculation rates compara-

Table 1. **LDF parameters on the last day of the treatment**

Parameters	CCLLI stage according to the Fontaine classification					
	Main			Control		
	II	III	IV	II	III	IV
Baseline perfusion rate (P.U)	1.52 ± 0.15	0.89 ± 0.18	0.42 ± 0.11	1.62 ± 0.16	0.98 ± 0.18	0.52 ± 0.14
Maximal perfusion rate (P.U)	6.20 ± 1.20	3.31 ± 0.36	0.83 ± 0.07	6.35 ± 1.12	3.42 ± 0.35	0.86 ± 0.06
σ (P.U)	0.93 ± 0.34	0.48 ± 0.12	0.16 ± 0.09	0.94 ± 0.39	0.49 ± 0.11	0.15 ± 0.08
Statistical difference p=0,56						

Table 2. **LDF parameters 30 days after the treatment**

Parameters	CCLLI stage according to the Fontaine classification					
	Main			Control		
	II	III	IV	II	III	IV
Baseline perfusion rate (P.U)	1.60 ± 0.13	0.96 ± 0.20	0.50 ± 0.10	1.59 ± 0.14	0.95 ± 0.20	0.48 ± 0.14
Maximal perfusion rate (P.U)	6.22 ± 1.20	3.31 ± 0.36	0.83 ± 0.07	6.16 ± 1.12	3.23 ± 0.35	0.76 ± 0.06
σ (P.U)	0.93 ± 0.34	0.49 ± 0.12	0.16 ± 0.09	0.90 ± 0.39	0.45 ± 0.11	0.14 ± 0.08
Statistical difference p=0,42						

Table 3. **LDF parameters 90 days after the treatment**

Parameters	CCLLI stage according to the Fontaine classification					
	Main			Control		
	II	III	IV	II	III	IV
Baseline perfusion rate (P.U)	1.79 ± 0.17	1.18 ± 0.16	0.73 ± 0.09	1.57 ± 0.16	0.92 ± 0.18	0.45 ± 0.11
Maximal perfusion rate (P.U)	6.91 ± 1.06	3.82 ± 0.31	0.96 ± 0.9	6.14 ± 1.01	3.20 ± 0.28	0.72 ± 0.08
σ (P.U)	0.98 ± 0.31	0.57 ± 0.13	0.24 ± 0.1	0.94 ± 0.28	0.51 ± 0.08	0.18 ± 0.09
Statistical difference p=0,067						

Table 4. **LDF parameters 1 year after the treatment**

Parameters	CCLLI stage according to the Fontaine classification					
	Main			Control		
	II	III	IV	II	III	IV
Baseline perfusion rate (P.U)	2.12 ± 0.31	1.39± 0.16	0.94 ± 0.11	1.45± 0.22	0.92 ± 0.16	0.42 ± 0.13
Maximal perfusion rate (P.U)	7.21 ± 1.11	4.19 ± 0.29	1.29 ± 0.13	5.99 ± 1.05	3.12 ± 0.26	0.73 ± 0.11
σ (P.U)	1.19 ± 0.30	0.66 ± 0.17	0.31 ± 0.09	0.99 ± 0.27	0.59 ± 0.13	0.25 ± 0.10
Statistical difference p=0,037						

ble to the original ones a year after the treatment. In contrast, the microcirculation rates were increased by 48.2% and myogenic vasomotor activity decreased by 24.3% one year after the AMSC treatment. The amplification of microcirculation could be explained due to a deeper modulation of the microcirculation vessels since it was increased by 2.1 times.

The LDF results indicate the active mechanism of tissue blood flow modulation compared to the passive mechanism of tissue blood flow modulation in the control group one year after carried out experiments.

The patient subgroups of the main group showed improved microcirculation parameters in case of following dosed physical exercises compared to patients who received AMSC therapy alone (significant difference p = 0.043). No other significant differences in microcirculation parameters between the patient subgroups of the main and control groups were found. Besides, there also was a significant increase in the perfusion rate among the patients who received AMSC therapy and followed dosed physical exercises compared to patients undergoing PgE1 treatment with dosed physical exercises in regard to the CCLLI stage (p = 0.04, p = 0.044, p = 0.048). The patients with the stage IV CCLLI, who received PgE1 treatment course following dosed physical exercises demonstrated a reduce in microcirculation parameters one year after as compared to the PgE1 treatment on its own because of patient`s inability to continue the exercises without symptoms of pain.

Distance walking study results. Based on the obtained data, we concluded that the average walking distance increased in patients who received AMSC treatment during one–year observation. On the contrary, patients who were given alprostadil showed a short period increase in the average walking distance. No significant difference in the average walking distance was found between the two patient groups. (p = 0.076).

Discussion

In spite of the positive results of the AMSC therapy in our study, the correlation of the results of the study with other groups of researchers, the important issues remain: the duration of the positive therapeutic effect after the start of treatment, the combination of this type of therapy with others, for example, stimulants of angiogenesis, and the possibility of reusing AMSC . Extremely important is providing of studies with a larger sample of patients and longer follow–up time after AMSC therapy. While stem cell therapy in patients with critical limb ischemia has been proven to be safe, feasible, and effective in smaller clinical trials , it is yet to reach its full potential as very few randomized and/or controlled trials have been completed using cell therapy. As such, larger and randomized clinical trials with more standardized methods of stem cell selection, delivery, and in vitro tracking are needed to establish cell therapy as a standard therapy for vascular regeneration. [9–12].

Conclusion

1. The results of this study found a significant difference in microcirculation improvement between patients receiving

AMSC and PgE1 treatment during the one–year observation period (p = 0.037).

2. A positive dynamic change of the microcirculation rate in case of PgE1 treatment is shown for 1.8 ± 0.3 months, whereas AMSC treatment demonstrates a significant increase in the microcirculation rate for 2.7 ± 0.4 months that was observed during the one–year period.

3. The combined AMSC therapy and dosed physical exercises have significantly increased the rates of microcirculation compared to the AMSC treatment alone (p = 0.043). Equally important, the combined PgE1 therapy and dosed physical exercises have insignificantly increased the rate of microcirculation in patients with the stage II, III CCLLI by 7% and 6.9%, respectively, however in patients with the stage IV CCLLI the microcirculation rate declined.

4. As far as the therapeutic effects of AMSC and PgE1 do not coincide with the time, both approaches should be considered as a complex treatment of CCLLI following dosed physical exercises.

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