ненная комбинированная сахароснижающая и липотропная терапия на фоне базовой терапии положительно повлияла на состояние гепатоцитов: уменьшилась активность АЛТ до (0,61±0,03) мкмоль/л, АСТ – (0,52±0,04) мкмоль/л, щелочной фосфатазы – (78,18±2,21) ед/л, гаммаглутамилтранспептидазы – до (52,34±1,26).

У всех больных достоверно уменьшились показатели цитолиза, мезенхимных-воспалительного синдрома, улучшились показатели гемограммы, стабилизировались показатели белкового и липидного обмена.

Ключевые слова: неалкогольная жировая болезнь печени, сахарный диабет 2 типа, комплексное комбинированное лечение.

COMPLEX APPROACH TO TREATMENT OF PATIENTS WITH NON-ALCOHOLIC STEATOGEPATITIS COMBINED WITH DIABETES MELLITUS TYPE 2

Pavlovskyi S. A.

Abstract. Non-alcoholic fatty liver disease (NAFLD) is becoming more and more an important cause of chronic liver disease. In generalizing numerous studies, data were found to confirm the association of NAFLD with type 2 diabetes or metabolic syndrome. Progression from fatty steatohepatosis to NASH occurs in 5-20% of patients with the possibility of developing liver fibrosis / liver cirrhosis. Patients with NASH and fibrosis patients should be identified as they risk mortality. Specific treatment of NASH is currently unavailable. Of importance is the definition of prognosis and optimal treatment of patients with NADH and directed monitoring of the development of hepatocellular carcinoma. Researchers offer complex therapy taking into account the main pathogenetic factors of NASH, which is increasingly combined with type 2 diabetes.

The goal is to optimize the treatment of patients with non-alcoholic fatty liver disease combined with type 2 diabetes. The study was conducted on 30 patients with NAFLD – at the stage of NASH. Control group – 20 healthy individuals. In order to identify the diagnosis of NAFLD, the data of clinical, laboratory, biochemical and instrumental studies were taken into account in full compliance with the standards of examination of patients with pathology of the organs of the gastrointestinal tract. The statistical processing of the obtained results was carried out using statistical statistic data package STATISTICA on Pentium-IV personal computer and application of parametric and non-parametric methods for estimating the obtained results. The ultrasound investigation revealed signs of fatty liver dystrophy – steatohepatosis (distal constriction of the signal, diffuse hyperhogenicity of the liver tissue, in comparison with the kidneys, and uncertainty of the contour of the vascular picture). In the refinement of the ultrasonographic picture of the liver, in the set of signs (slight increase in echogenicity, visualization of the wall of the mediums and large caliber veins) in 5 patients (16.7% of cases), I was diagnosed as stage fatty liver disease. Moderate elevation of echogenicity of the liver, visualization of only partial and segmental veins, corresponding to the II stage of hepatatosis, was detected in 15 patients (50.0%) cases. In the 10 patients (33.3%), III stage of fatty hepatosis was visualized. The reliability of the difference in values between independent quantitative values was determined with a normal distribution according to Student's criterion, and in other cases, using the Mann-Whitney U-criterion. With the use of combined treatment with combined hypoglycaemic (diabetic and pioglitazone) and lipotropic (heptral) therapy, the results of treatment showed a significant improvement in the subjective and objective state of patients. Pain syndrome remained tangible (1.8 times fewer patients than prior to treatment); dyspeptic syndrome – decreased by 1,7 times, appetite decreased – (a decrease of 1,9 times), astenovegetative syndrome – (a decrease of 1,9 times). The applied combined hypoglycemic and lipotropic therapy on the background of basic therapy positively influenced the state of hepatocytes: the activity of ALT decreased to (0.61±0.03) μmol/l, AST (0.52±0.04) μmol/L, alkaline phosphatase – (78.18±2.21) units/l, gammaglutamyltranspeptidase – up to (52.34±1.26).

In all patients, cytolysis, mesenchymal-inflammatory syndrome significantly decreased, hemogram rates improved, and lipid and metabolic parameters were stabilized.

Key words: non-alcoholic fatty liver disease, diabetes mellitus type 2, complex combined treatment.

Рецензент – проф. Бобирьова Л. Є.

Стаття надійшла 11.05.2018 року

DOI 10.29254/2077-4214-2018-2-144-221-224

UDC 615.361.013.85.014.41: 57.085.23

[1]Prokopiuk V. Yu., [1]Falko O. V., [2]Karpenko V. G., [1]Chub O. V., [2]Loginova O. O.

INFLUENCE OF NATIVE AND CRYOPRESERVED PLACENTAL DERIVATIVES ON THE SPLENOCYTE FUNCTIONAL CHARACTERISTICS IN VITRO

[1]Institute for Problems of Cryobiology and Cryomedicine of NAS of Ukraine (Kharkiv)

[2]Kharkiv Medical Academy of Post-Graduate Education (Kharkiv)

v.yu.prokopiuk@gmail.com

Publication relation to planned scientific research projects. This study was supported by the Institute for Problems of Cryobiology and Cryomedicine of NAS of Ukraine, within Project No. 0114U001319 «Investigation of the geroprotective and gerotherapeutical effect of placental bioobjects».

Introduction. Autoimmune diseases affect 3-5% of population in the world. The most common pathology are multiple sclerosis, type I diabetes, autoimmune hepatitis, biliary cirrhosis, ulcerative colitis, and rheumatoid arthritis. They often lead to a significant life`s quality deterioration and requirement of long-term application of medical therapy: hormonal and cytostatic drugs with nonspecific immunosuppressive and antiproliferative activity. Typical side effects are increased susceptibility

to infection, total toxicity, osteoporosis, myelotoxicity, oncological risks, metabolic disorders [1,2].

The promising direction of autoimmune pathology treatment is the using of stem cells and cytokines that they produce [2,3]. The effectiveness of autoimmune diseases treatment with mesenchymal stem cells (MSCs) has been experimentally proved [1]. When comparing immunomodulatory activity of cells from different sources, the major activity of placental derivated cells was proved [4]. The effect of placental derivatives is natural, since tolerogenic changes in the immune status and regression of autoimmune pathology are typical for pregnancy [5]. The same properties have different placental components (cord blood serum, cells, explants, extract) [6,7,8]. For the successful placental derivatives clinical application, it is necessary to establish a suitable low-temperature bank, because cryopreservation is the only possible method of their storage [9]. At the same time, low temperatures can affect bioobjects, changing their activity [10]. Despite the large number of studies on immunomodulatory MSC `s and pregnancy`s action, the effects of native and cryopreserved placental derivatives on immunocompetent cells are unclear.

The aim of the work was to compare the effect of freshly isolated and cryopreserved placental derivatives on splenocytes.

Object and methods. To achieve this aim, the effects of media, conditioned by freshly isolated placental cells (PC), cryopreserved cells (CPC), freshly-isolated placental explants (PE), cryopreserved placental explants (CPE), and medium, with 10% of placental extract (E) on isolated mouse splenocytes were studied. Splenocytes were cultured in medium for one day, metabolic activity was evaluated by MTT test and the functional activity was assessed by blast transformation reaction.

Full term normal human placentas were collected after an informed consent. Cultures from three different placentas were used. PE were obtained by the previously described method: placental villi were separated with a size not more, then 3 mm [11]. PC were isolated by the enzymatic method, described earlier, using 0.25% trypsin («BioWest», France), the characteristic of the CP as MSC was also performed previously [9].

PC and PE were cryopreserved, according to the previously described program [9,11]. As cryopreservation medium there was used Dulbecco's Modified Eagle Medium with high glucose and L-glutamine (DMEM) («BioWest», France), 10% fetal bovine serum (FBS) and 10% dimethylsulfoxide («Sigma», USA). Samples were frozen in cryotubes («Nunc», USA) at a rate of 1°C/min down to -70°C using Mr.Frosty™ Freezing Container containers («Thermo Fisher Scientific», USA) filled with isopropanol, followed by immersion in liquid nitrogen. Thawing was carried out in a water bath at 37°C.

To obtain media, conditioned by PE and CPE, 10 mg of cryopreserved or freshly-isolated PE were cultured for 1 day in 1 ml DMEM in a CO_2 incubator («Thermo Fisher Scientific», USA) at 37 ° C in an atmosphere with 5% CO_2 in 24-well plate («SPL», Korea). To obtain media, conditioned by CP and CPC, cells were cultured until monolayer (about 1×10^6 per 5 ml of medium on 25 cm^2 flaks («SPL», Korea)), the medium was changed, cultured for 1 day in DMEM at 37°C in 5% CO_2.

The extract was obtained by cryodestruction-cryoextraction method: three cycles of freezing-warming of chopped placental tissue in a water-salt solution, followed by debris` separation by centrifugation, supernatants were removed into sterile cryotubes and frozen by direct immersion in liquid nitrogen.

Splenocytes were isolated from spleens of BALB/c mice. The spleens were removed, chopped, filtered through a 100 μm cell filter, the cell suspension was washed, resuspended in DMEM with 10% FBS and antibiotic-antimycotic.

For the MTT test, 1×10^5 cells per well of 96-well plate were taken. MTT (Sigma, USA) was added at a final concentration of 0.5 mg/ml, incubated for 4 hours. Then, the medium was removed carefully, the formazan crystals were dissolved in 10% SDS solution in dimethylsulfoxide. Absorption was measured on a plate reader SM600 («Utrao», China) at 570 nm. Each experiment was repeated in 8 samples of three different cultures.

Blasttransformation reaction (BTR) was carried out according to a standard microcultural method with phytohaemagglutinin [12], with the difference that the RPMI medium was replaced by DMEM, conditioned by the placental derivatives. Splenocytes were resuspended in DMEM to a concentration of 5×10^6 cells in 1 ml, cultivated for 48 hours with the addition of 0.007 ml of 2-Me phytohemagglutinin per 10 ml of medium. The number of blast cells per 250 cells was evaluated morphologically using Romanowsky staining.

Mann-Whitney U-criterion tests were performed for comparison of groups. Data were analyzed using «Past

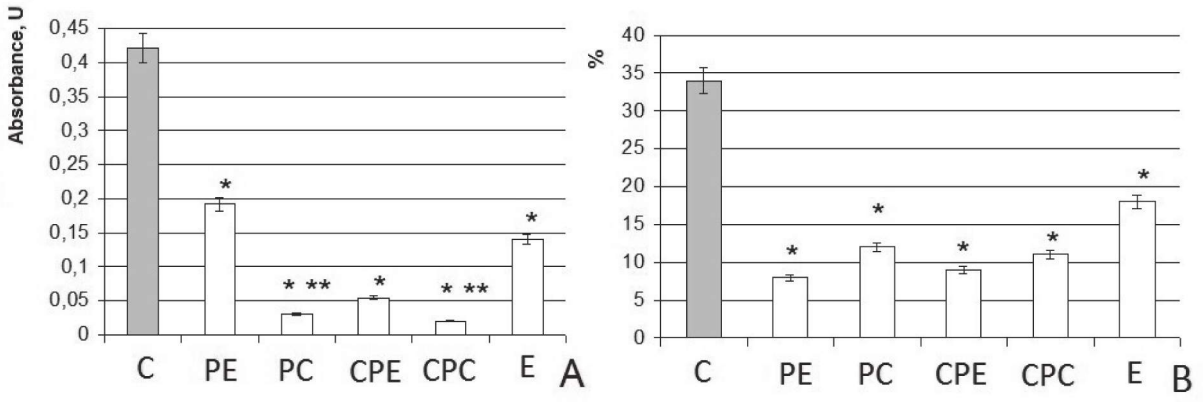

Fig. 1. Activity of splenocytes` culture: A – MTT test, B – RTB. C – control (culture not conditioned by the placental derivatives), PE, PC, CPE, CPC, E – media, conditioned by the placental derivatives. * – difference is statistically significant with control p <0.05, ** – difference is statistically significant with freshly-isolated objects p <0.05.

V.3.15» software (University of Oslo, Norway). All experiments were approved by bioethics committee of the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine (protocol No. 2, June 3, 2013), according to the "General Principles of Animal Experiments", Approved by the V-th congress in Bioethics (Kyiv, 2013) and the «European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes» (Strasbourg, 1986).

Results and discussion. Splenocytes were isolated successfully. Microscopic examination after incubation with MTT showed, that formazan crystals were only in splenocytes but not in the erythrocytes. It allows using the MTT reaction without traumatic procedure of erythrocytes removing.

MTT reaction showed that cultivation of splenocytes with media conditioned by the PC, CPC, EP, CPE, reduce the metabolic activity of splenocytes **(Fig. 1, A).** The suppressive activity was similar for both freshly-isolated and cryopreserved objects. The effect of cryopreserved placental cells and explants was slightly but significantly higher than the effect of native ones. When studying BTR, reduction of cells transformation in blast forms after the action of media, conditioned by placental derivatives **(Fig. 1, B)** was shown. The MTT characterizes the mitochondrial function and overall cellular metabolism, while BTR characterizes specific immunity.

Mechanisms of immunomodulatory effects of placenta and its derivatives are explained by the impact of humoral factors: cytokines, hormones, chorionic gonadotropin [1,3,8].

In previous studies the effectiveness of placental bioobjects in encephalomyelitis, rheumatoid arthritis, antiphospholipid syndrome, lupus erythematosus, and Crohn's disease was shown. The use of placental objects in infectious pathology increases the number of complications, which may also be the result of immunosuppression [7]. The obtained data on placental bioobjects` immunosuppressive effects on isolated cells indicates the direct effect of placental humoral factors on immunocompetent cells. This is also confirmed by the similar effect of media conditioned cells, placenta explants, and the action of the placenta extract.

Increased activity of the medium conditioned with cryopreserved placental cells and explants may indicate the bioobject`s activation after thawing, or cryoselection of certain cell types [10].

The obtained data and analysis of the literature demonstrate the perspective of placental derivatives application in the treatment of autoimmune diseases but not in acute infectious diseases.

Conclusions. Media, conditioned by placental cells, explants and placental extracts reduce the splenocytes` metabolic activity and the activity of the blasttransformation reaction.

Media, conditioned by cryopreserved placental cells and explants reduce the splenocytes metabolic activity more significantly than the media, conditioned by freshly isolated ones.

Perspectives for future researches are to compare the mechanisms of placental derivatives immune modulating effects with MSCs, isolated from other sources in model experiments.

Conflict of interest statement. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ВПЛИВ НАТИВНИХ ТА КРІОКОНСЕРВОВАНИХ ПОХІДНИХ ПЛАЦЕНТИ НА ФУНКЦІЙНІ ХАРАКТЕРИСТИКИ КУЛЬТУРИ СПЛЕНОЦИТІВ IN VITRO

Прокопюк В. Ю., Фалько О. В., Карпенко В. Г., Чуб О. В., Логінова О. О.

Резюме. На аутоімунні захворювання страждають близько 3-5% людей у світі. Застосування мезенхімальних стовбурових клітин та плацентарних біооб'єктів розглядаються як перспективний, нетоксичний метод лікування. Метою роботи було порівняння впливу свіжовиділених та кріоконсервованих похідних плаценти на спленоцити. Спленоцити мишей культивували з середовищами, кондиційованими нативними

та кріоконсервованими клітинами та експлантами плаценти, з середовищем, що містить 10% екстракту плаценти. Виявлено, що середовища, кондиційовані клітинами, експлантами плаценти, та екстракт плаценти знижують метаболічну активність спленоцитів та активність реакції бласттрансформації лімфоцитів. Середовища, кондиційовані кріоконсервованими клітинами та експлантами плаценти більше знижують метаболічну активність спленоцитів, ніж, середовища, кондиційовані свіжовиділеними біооб'єктами.

Ключові слова: спленоцити, культура, плацента, клітини, екстракт, кріоконсервування.

ВЛИЯНИЕ НАТИВНЫХ И КРИОКОНСЕРВИРОВАННЫХ ПРОИЗВОДНЫХ ПЛАЦЕНТЫ НА ФУНКЦИОНАЛЬНЫЕ ХАРАКТЕРИСТИКИ КУЛЬТУРЫ СПЛЕНОЦИТОВ IN VITRO

Прокопюк В. Ю., Фалько О. В., Карпенко В. Г., Чуб О. В., Логинова О. А.

Резюме. Аутотимунными заболеваниями страдают около 3-5% людей. Использование мезенхимальных стволовых клеток и плацентарных биообъектов рассматриваются как перспективный, нетоксичный метод лечения. Целью работы было сравнение влияния свежевыделенных и криоконсервированных производных плаценты на спленоциты. Спленоциты мышей культивировали со средами, кондиционированными нативными и криоконсервированными клетками и эксплантами плаценты, со средой, содержащей 10% экстракта плаценты. Выявлено, что среды, кондиционированные клетками, эксплантами плаценты, и экстракт плаценты снижают метаболическую активность спленоцитов и активность реакции бласттрансформации. Среды, кондиционированные криоконсервированными клетками и эксплантами плаценты, сильнее снижают метаболическую активность спленоцитов, чем, среды, кондиционированные свежевыделенными биообъектами.

Ключевые слова: спленоциты, культура, плацента, клетки, экстракт, криоконсервирование.

INFLUENCE OF NATIVE AND CRYOPRESERVED PLACENTAL DERIVATIVES ON THE SPLENOCYTE FUNCTIONAL CHARACTERISTICS IN VITRO

Prokopiuk V. Yu., Falko O. V., Karpenko V. G., Chub O. V., Loginova O. O.

Abstract. Autoimmune diseases affect about 3-5% of people. The mesenchymal stem cells and placental bioobjects application is a promising, non-toxic method of treatment. The mechanism of the influence of native and cryopreserved placental derivatives on immunocompetent cells remains unclear.

The aim of the work was to compare the effect of freshly isolated and cryopreserved placental derivatives on splenocytes.

Object and methods. Mouse splenocytes were cultured in media conditioned with native and cryopreserved placental cells and explants, with a medium containing 10% of the placental extract. The metabolic activity of splenocytes was assessed by MTT test, functional activity was assessed by the blasttransformation reaction.

Results. It has been shown that the cells conditioned by placental cells, explants, and placenta extract reduce the metabolic activity of splenocytes and the activity of the blasttransformation reaction. The media, conditioned by cryopreserved placental cells and explants, reduce the metabolic activity of splenocytes in a bigger extent, than those that are conditioned with freshly isolated objects.

Conclusions. Various placental derivatives application is promising treatment of autoimmune diseases, but not acute infectious diseases.

Key words: splenocytes, culture, placenta, cells, extract, cryopreservation.

Рецензент – проф. Білаш С. М.
Стаття надійшла 17.05.2018 року

DOI 10.29254/2077-4214-2018-2-144-224-228
УДК 616.152.21-053.31-07-08

Садыгова Ш. А.

ДИНАМИКА МИКРОЭЛЕМЕНТОВ В КРОВИ У НОВОРОЖДЕННЫХ, ПЕРЕНЕСШИХ ПЕРИНАТАЛЬНУЮ АСФИКСИЮ

Азербайджанский Медицинский Университет (г. Баку, Азербайджан)
nauchnayastatya@yandex.ru

Связь публикации с плановыми научно-исследовательскими работами. Данная работа является фрагментом выполняемой диссертации на соискание ученой степени доктора философии по медицине «Состояние гомеостаза и метаболического статуса у новорожденных, перенесших перинатальную асфиксию».

Вступление. Несмотря на достижения, полученные в последние годы в области перинатологии и неонатологии, перинатальные поражения центральной нервной системы (ЦНС) продолжают оставаться основной причиной перинатальной и неонатальной смерти.

Согласно сведениям Всемирной организации здравоохранения, у 10% населения детского возраста выявляются нервно-психические расстройства, и причиной до 80% из них является перинатальное поражение ЦНС различного происхождения [1-3].

Согласно сведениям Американской Педиатрической Академии летальный исход при тяжелой перинатальной асфиксии (ПА) происходит в ранний неонатальный период в 50-60% случаев [4]. Также было установлено, что до 70% случаев основной причиной инвалидности у детей является пре- и перинатальная патология [5,6].

Поэтому в настоящее время, одной из самых важных проблем, стоящих перед перинатологией и