**Abstract**

Currently, a fairly wide range of studies is devoted to studying the possibilities of using placenta extracts obtained by lysis of human placental tissues, sheep, goats, cattle and horses in medicine. Based on these data, purpose to our study was to explore ix biological properties of the active drug based on horse placenta under conditions in vitro. Research methods: percoll density gradient isopycnic centrifugation, trypan blue exclusion test, MTT test, cell culture, light microscopy, flow immuno-cytofluorimetry, enzyme-linked immunosorbent assay, proliferation test using intravital fluorescent CFSE stain. Bytes iopreparat s based horse placenta no cytotoxic th action on human mononuclear cells of peripheral blood at 24 hours exposure to concentrations not exceeding 3,250 pg / ml. and have a cytoprotective effect against mononuclear cells in human peripheral blood, reducing the intracellular level of reactive oxygen species after short-term exposure. The drug also induces comfort production of the anti-inflammatory cytokine IL-4 by mononuclear cells of human peripheral blood.

**Key words:** Placenta, Immunomodulator, T-lymphocytes, Proliferation.

**Introduction**

The placenta is an important organ that during intrauterine development provides the relationship between the mother and fetus, and also performs respiratory, excretory, trophic, protective, endocrine and immune functions. Currently, a fairly wide range of studies is devoted to studying the possibilities of using placenta extracts obtained by lysis of human placental tissues, sheep, goats, cattle and horses in medicine. Such extracts do not contain cells, but possess a wide range of proteins, minerals, amino acids, and steroid hormones [1]. According to various research groups, such extracts have anti-inflammatory, analgesic [2],
antioxidant [3, 4], cyto- and radioprotective [5], anti-allergic properties [6-8], and also stimulate proliferation and repair processes [9, 10].

Most of the research is devoted to the study of the biological properties of extracts obtained from human placenta. So it was shown that placenta extracts enhance the proliferation of fibroblasts and cord blood cells in vitro [7, 10]. The cytoprotective and antioxidant properties of the extracts are associated with protein components; in particular, with alpha-fetoprotein om [3, 4]. Model animal studies have shown that the introduction of extracts increases the resistance of animals to oxidative stress [11]. Placental extracts reduce the concentration of free radicals and proinflammatory cytokines IL-6, TNF, and IL-1, while increasing the formation of progenitor cell colonies and reducing oxidative and radiation damage to cells in vitro [5, 11]. Biosafety analysis of extracts of pork placenta revealed the absence of toxic or mutagenic effects on cell cultures and models of adult animals; however, fetotoxicity in animals in early pregnancy has been reported [12].

In the clinic expressed placental extracts beneficial effects were obtained in treatment of wounds, non-healing ulcers and burns, wherein the receptacle achitelno Expand va familiarize rate of epithelialization observed decrease in infiltration by leukocytes and the reduction of pain [13]. The mechanism of action of placenta extracts during wound healing is explained by an increase in the level of TGF \( \beta \) in the early phase of regeneration and VEGF in the late phase, increased angiogenesis and increased expression and CD31, as well as the presence of FGF in placental extracts [14, 15]. Experimental studies of the effect of placenta extracts on the behavior and physical condition of animals showed a decrease in symptoms of fatigue and increased resistance to physical activity [16], which was explained by an increase in intracellular calcium, activation of splenocytes and T cells, as well as a decrease in the synthesis of proinflammatory cytokines associated with fatigue (IL-6, TNF, and IFN\( \gamma \)) [16]. Similar results were obtained in preclinical studies [17].

Placental extracts have been shown to be highly effective in neurology, supporting the regeneration of nerve tissue in the experimental treatment of nerve damage and facial spasm. The authors explain the effect obtained by increased synthesis of regenerative factors GAP-43 and Cdc2 after exposure to placental extracts [18, 19]. Placental extracts were effective in the treatment of rheumatoid arthritis [20] and experimental renal failure [21]. A certain amount of practical experience with the use of placenta extracts has also been accumulated in veterinary medicine. Here, the extracts were used to stimulate mammogenesis, lactogenesis, and galactopoiesis [22].

Previously been rendered placental extracts immunomodulating properties [23-25]. Thus, Chinese researchers isolated and purified several fractions of immunomodulating peptides from a water-soluble extract of cow placenta and characterized their in vitro effect on lymphocyte proliferation [23]. The immunomodulating activity of the extract of pork and horse placenta on mouse lymphocytes was demonstrated [24, 25]. It was suggested that peptides with a molecular weight of up to 10 KDa are the main bioactive fraction of the placenta with immunomodulating and antioxidant effects [26].

With respect to the extracts obtained from placentas s animal studies Nia placenta sheep are the earliest and most widely used. In connection with the success of the study of the placenta of sheep, more attention is being paid to studying the possibilities of using for medical purposes extracts from the placenta of other animals (for example, cattle, pigs, etc.). Placenta horses is in currently the least explored. Taking into account national peculiarities and traditions of the peoples of Kazakhstan, where the horse is a traditional source of food and folk medicinal resources, it seems suitable th explore the placenta horses and scientifically substantiate its healing properties. It should be noted that the use of the placenta of horses does not harm the environment and does not upset the ecological balance, as it relates to natural waste. Thus, the aim of this study was to assess the toxicology and study the immunomodulating effect of an aqueous extract of equine placenta on human peripheral blood mononuclear cells.

**Research materials**

The receipt of material and conservation of the horse placenta for research was taken from the farms of Akmola and Karaganda regions that are on ecologically clean food. The placenta was taken after delivery from mares immediately after the vaginal delivery, the umbilical cord and amnion were disposed of. The remaining tissue was washed thoroughly with ice-cold phosphate-saline buffer to completely remove all traces of blood.
Placental tissues were destroyed using a tissue homogenizer (Tissue Tearor, Biospec Products Inc., Bartlesville, OK, USA) in cold phosphate-buffered saline. Tissue homogenates were centrifuged at 6000 g for 15 minutes and the supernatants lyophilized. Placenta extracts were sterilized using $\gamma$-radiation into a room using a source of cobalt-60 isotopes using ILU-10 accelerators (Russia) at the Institute of Nuclear Physics in Almaty, Kazakhstan. The absorbed dose of $\gamma$-irradiation was 30 kGy at a speed of 1 kGy / h.

Figure 1 - biological preparations obtained on the basis of horse placenta after centrifugation at 6000 g for 15 min.

In the experiments we used samples of venous blood obtained from volunteer donors (cf. age 39.6 ± 1.3, 5 years (range 19-72), n = 16; women: cf. age 42.2 ± 2.1, 7 years, n = 6; men: cf. age 37.7 ± 5.7 years, n = 10) who do not have obvious signs of any disease. Venous blood was taken from the ulnar vein of healthy volunteers in a volume of 9-18 ml into sterile tubes with EDTA (VenoSafe) with a nominal volume of 9 ml using a Venosafe Quick Fit holder. The study was approved by LEK JSC "National Medical University" (extract from protocol No. 7 (84) of June 10, 2019). All donors gave informed consent, the study was conducted in accordance with the ethical principles of the Helsinki Declaration.

The following reagents were used during the experiments: culture medium RPMI-1640, L-glutamine (L-glutamine), streptomycin / penicillin, fetal bovine serum (FBS), bovine serum albumin (BSA), trepan blue, histopack-1077, percoll, dimethyl sulfoxide (DMSO), phosphate-buffered saline (PBS) MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide), to onkanavalin A (ConA), LPS Salmonella typhimurium, PHA, CFSE (Sigma-Aldrich, Germany), propidium iodide (BD Pharmigen, USA), 2'-7'-dichlorofluorescein diacetate (DCFDA), Interleukin-4-IFA-Best, Interleukin-1-IFA-Best (Vector-Best, Russia).

**Research methods**

PBS, is used for the preparation of washing buffer solution was prepared by dissolving 0.005 M NaH 2 PO 4 and 0.15 M NaCl in distilled water, adjusting the pH to 7.4 with pomoschyu 3M NaOH. All the resulting solutions were sterilized by passage through a sterile membrane filter having a pore diameter of 0.22 micron, and stored in a refrigerator at + 4-8 oC until use.

The RPMI-1640 liquid culture medium was prepared from a dry preparation, dissolving it in deionized water and adjusting the pH to 7.4, according to the recipe of the manufacturer. The final product was sterilized by ultrafiltration through a sterile membrane filter with a pore diameter of 0.22 μm using a peristaltic pump. Complete culture medium (PCB) was prepared based on RPMI-1640 supplemented with 10% FBS, 100 μg / ml penicillin / streptomycin and 2 mM glutamine.

Determination of protein concentration in the obtained samples of the placenta extract was determined spectrophotometrically by measuring the optical density of the solution (absorption) at 280 nm and 260 nm. An aliquot of the biological product was dissolved in PBS (dilution 1:60), the solution was placed in a cuvette and the optical density was measured on a spectrophotometer relative to PBS.

To isolate mononuclear adhesive current peripheral blood (PBMCs), 10 ml of whole blood and 10 ml layered on histopaka-1077 or Percoll appropriate density and centrifuged for 3 0 min at 400 g at 20 for S.
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Interphase ring mononuclear cells were washed 2 times 20x the volume of medium RPMI-1640 at 20 g for 15 min at 20 about C.

The MTT test was used to assess the toxic effect of biopreparations based on horse placenta extracts on the viability of PBMCs. For this, PBMCs were cultured in 96-well plates at a concentration of 1 x 10^5 / well in 200 ul PKS for 24 hr. At 37 of C and 5% CO 2. Biological products based on horse placenta were introduced in final protein concentrations of 1500, 750, 375, 187.5, 93.75, 46.88 μg / ml. Controls were cultures of PBMCs that were incubated in the SCC. At the end of incubation, each well was added 20 μl of MTT solution (5 mg / ml) and further incubated for 4 hours at 37 to C. W ATEM supernatant was removed and the precipitate was dried at room temperature and the formed formazan crystals were dissolved in 100 ulDMSO. The optical density was measured on an immunoassay analyzer at 492/630 nm.

The cytotoxicity index (C) was calculated using the following formula:

\[ C (\%) = \left( \frac{K - O}{K} \right) \times 100 \]

where O is the optical density in the experimental cell containing MNP K and a biological product, K is the optical density in the control cell containing only MNPK.

Cytotoxicity Biopros Ratov based horse placenta was also evaluated by inclusion PI. PBMCs were cultured in 10% of SCC in a concentration of 5 x 10^5 / ml in the presence of various concentrations of a biological preparation in a final volume of 1 ml at 24 and 72 hr. At 37 of C and 5% CO 2. The cells were then washed with PBS at 300 g for 5 min and resuspended in flow cytomlimeter. PI was added according to the instruction of the manufacturer, incubated for 1 min in the dark and then analyzed on a flow cytometer FACS Calibur (BD Biosciences, USA), using I FL -2. Data analysis was performed using programnogo ensure CellQuest Pro (BD Biosciences, USA). As a control, I used unpainted cells. Analyzed at least 50 thousand cells. Lymphocytes and monocytes gated on the FSC vs SSC Dotplot, which reflects the parameters of direct and lateral light scattering and allows us to judge the morphology of cells. The inclusion of PI indicated a loss of membrane integrity and death and cells.

Succinimidyl ester Carboxyfluorescein( CFSE ) - Intravital Single nonfluorescent Single label. Influenced in nutrikletochny x esterases in living cells CFSE converted into fluorescent molecule which reacts with intracellular free amines to form a covalent stable conjugates, dye-protein. The conjugate of the dye with proteins that forms in labeled cells is retained by these cells for several fission cycles. Therefore, CFSE is used to evaluate cell proliferation.

To assess the effect of a biological product for T cell proliferation, PBMC obtained from volunteer donors, resuspended in RPMI-1640, was added a solution of CFSE (5 mM) and incubated for 6 min at 4 o C. The cells were washed twice with cold thPBS. Labeled CFSE cells were cultured in PKC in the presence of 5 μg / ml ConA at 37 ° C and 5% CO 2 at a final concentration of 2 x 10^6 cells / ml. As a control, a controlling th sample s without the addition of a biological product. After 72 hours, the cell culture was washed and the level of T-cell proliferation was assessed by diluting CFSE on a flow cytometer.

The antioxidant activity of the biological product was evaluated in a test with DCFDA (2′-7′-Dichlorodihydrofluorescein diacetate) by flow cytometry. DCFDA is a stable, non-fluorescent compound that, upon penetration into the cell, is converted to DCF by intracellular esterases. The loss of the acetate group leads to the oxidation of the ROS product and its conversion to highly fluorescent 2′, 7′ dichlorofluorescein (DCF), which when excited at 488 nm emits green fluorescence proportional to the intracellular level of ROS. Thus, the change in DCF fluorescence reflects the intracellular accumulation of ROS. Stock solution DCFDA (5mM) were prepared in DMSO and stored at -20 for C.

The influence of biological products on the production of ROS of human MNPK was studied according to the previously described method [28]. Briefly, the resulting MNPCs were resuspended in 10 % PKC at a concentration of 5 x 10^5 cells / ml and incubated for 24 hours at 37 °C and 5% CO 2. Then the cells were washed with PBS, pre-treated with 2 μM DCFDA in serum-free medium for 1 h. After incubation, the cells were washed from excess DCFDA PBS and incubated in serum-free medium with the addition of various.
concentrations of the biological product for 2 hours. Cells cultured without biological product. Then the cells were washed and immediately analyzed on a flow cytometer.

The effect of the biological product on the level of secretion of IL-1β, IL-4 MNPK was analyzed using ELISA using commercial kits (Vector-Best). The level of IL-1-1 MNPK secretion in the culture supernatants was evaluated after 24 h ASF stimulation of LPS cells (200 ng/ml).

**Statistical data processing**

The obtained data were processed using mathematical statistics methods on a personal computer using the Excell and Prizm 6 software (Graph Pad) application programs. Design data flow cytometry was performed using software Flowing software 2.5.1. R figures contain information in the form of arithmetic means (M) and standard deviation (SD). The significance of differences in mean values between the two experiments was calculated by Student's t test (TTEST). Where indicated, the results were analyzed using one-way ANOVA analysis followed by a Tukey test. Paizichii I considered insignificant and, if the probability of a null hypothesis of no more than 5% (p = 0.05).

**Results**

Toxicological assessment biopreparation based horse placenta under conditions in vitro. The protein concentration in the obtained supernatants was determined spectrophotometrically by measuring the optical density of the solution (absorption) at 280 nm and 260 nm. The measurement was performed three times. According to the obtained result, the protein concentration in the samples was 5.34 mg/ml. The samples were then aliquoted in the amount of 300 μl and stored until use at -20 for C

A study of the cytotoxic effect of horse placenta-based biologics was performed on mononuclear cells of healthy donors in an MTT test that measures the number of viable cells by determining the activity of NADH-dependent dehydrogenase. Biological preparations based on horse placenta were introduced in final protein concentrations of 1500, 750, 375, 187.5, 93, 8, 46, 9 μg/ml. As a control, cells incubated in 10% PKC were used.

During the study, we observed that the addition of MTT after 24 h of cell incubation in the presence of biological products led to very rapid intensive formation of formazan crystals in the culture fluid regardless of the presence of cells (Fig. 2), which was probably due to the ability of the biological products to restore MTT.

![Figure 2 - Conducting a standard MTT test during the cultivation of MNPK in the presence of biologics based on horse placenta.](image)

In the next stage of operation, etc. To assess the cytotoxic activity of biological products, we used the test to include propidium iodide (PI), membranoneprontisemogo dye that does not stain the living cells, but at the same time penetrating a through damaged membranes of dead and apoptotiruyuschih cells and tying etsya with their double-stranded DNA. PBMC obtained from healthy donors were cultured in the presence of times personal concentrations biopreparation s for 24 h and 72 h and more cell death was evaluated by inclusion PI by flow tsiotoflumetr. When biologics and PCB were added to the test tubes, special attention was paid to the preservation of 10% FBS concentration. Cells cultured in 10% PKC were used as a control.
Flow Cytometry results are shown in Figure 4.

Figure 4 - Cytotoxicity of biopreparations based on equine placenta in relation to human peripheral blood lymphocytes in the PI inclusion test. MNPKs were cultured in the presence of various concentrations of biopreparations based on horse placenta for 24 hours and 72 hours, then the cells were washed, labeled with PI and analyzed on a flow cytometer. Figure A presents the generalized data (M ± SD), n = 5. Figure B shows representative histograms of one of 5 independent experiments for PBMCs after cultivation with biologics for 72 hours.

Significance of differences is presented as * p <0.05 compared with the corresponding control. K unstained - cells cultured in PCB without the addition of drugs, unpainted PI, K - cells cultured in PCB without the addition of drugs, stained with PI.

From figure 4 it is seen that no cytotoxic biologics the action on PBMCs after 24 hours. The cell culture regardless of the selected concentration.

The study of antioxidant activity of biological products based on horse placenta under conditions in vitro

To assess the antioxidant activity of biological products based on horse placenta, we determined the redox status of MNPK by the level of intracellular ROS after 2 hours of incubation in the presence of biological products. ROS levels were measured by flow cytometry using DCFDA. MNPCs were treated with DCFDA for an hour, then the cells were washed and incubated in serum-free medium with the addition of biological products for 2 hours. The averaged values showing the percentage of cells positive for DCFDA expression, which directly correlates with the ROS content in the cells, are shown in Figure 5. From Figure 5 it is seen that 2 hour incubation in the presence of biological preparations at concentrations 1500 mcg / ml and 375 ug / ml significantly reduced the level of ROS in human PBMCs when compared to cells incubated in the PCB without biologics.

The significance of differences is presented as * p <0.05 and ** p <0.005 compared to the control.
Figure 5 - Production of human ROS MNPK after 2 hours of incubation with biopreparations based on horse placenta. The figure shows the generalized data (M ± SD), n = 8.

Thus, biologics significantly improved the redox status of cells with short-term exposure. As far as we know, this is the first study on the effect of placental extracts on the level of intracellular ROS in human MNPK. Studying the influence of biological products on the basis of horse placenta on mitogen-stimulated T cell proliferation in conditions in vitro.

To assess the immunomodulatory activity of a biopreparation based on horse placenta for cellular immunity, we evaluated the mitogen-induced blastogenic response of PBMCs when cultured in the presence of a biological product. 3 concentrations were selected not toxic to PBMCs: 1500 pg/ml, 375 micrograms/ml and 46.9 mcg/ml. Concanavalin A (Con A), a widely used antigen-independent mitogen that binds mannose residues of cell surface glycoproteins, including the T cell receptor, and leads to polyclonal proliferation of T cells, was used as a mitogen [40]. As control, MNPKs cultivated in PKC without the addition of a biological product were used. Proliferation was assessed on a flow cytometer by decrease luminescence CFSE, Kotor th pre-labeled cells. As control in the analysis on a flow cytometer, MNPK, unlabeled CFSE were used. The results were analyzed using one-way ANOVA analysis of the variance followed by the Tukey test. The results are presented in Figure 6.
Figure 6 - Effect of a biopreparation based on horse placenta on mitogen-induced proliferation of T cells of human peripheral blood. MNPKs preliminarily labeled with CFSE were cultured in the presence of Con A and various concentrations of biologic preparation No. on the basis of horse placenta for 72 h, then the cells were analyzed on a flow cytometer. Figure A presents the generalized data (M ± SD), n = 4. Figure B shows representative histograms of one of 4 experiments. Figure B shows microphotographs of MNPK under light microscopy (x200).

The significance of differences is presented as * p <0.05. K unstained - cells cultured in PKC without the addition of drugs, unpainted CFSE; K - cells cultured in PKC without the addition of drugs stained with CFSE; K + ConA - cells cultured in PKC with Con A (5 μg/ml) without the addition of preparations stained with CFSE.

The figure shows that the biological product significantly inhibited the proliferation of T cells at concentrations of 1500 μg/ml and 375 μg/ml compared to the control.

Studying the effect of horse placenta-based biologics on the cytokine-producing activity of human PBMCs in vitro

To study the possible effect of horse placenta extracts on the cytokine-producing activity of PBMCs, we examined the level of secreted IL-4 and IL-1 in cell supernatants after 24-hour exposure to biological products added at final concentrations of 1500 μg/ml, 375 μg/ml and 46.9 mcg/ml. MNPKs cultivated without biologics were used as a control.

The results of a study of the effect of biologics on IL-4 production are presented in Figure 7. 24-hour incubation in the presence of biologics induced a dose-dependent increase in the production of IL-4 PBMCs in sheep compared to the control.

Figure 7 - Effect of biologics based on horse placenta on the production of IL-4 MNPK human. PBMCs were cultured in the presence of various concentrations of biologicals based on horse placenta 24 hours, the supernatant was collected and analyzed by IL-4 content by immunoassay. The figure shows the generalized data, n = 2.
The results of a study of the effect of biological products on IL-1β production are presented in Figure 8. The level of spontaneous production of human IL-1β MNPK after 24-hour exposure to biological products, as well as the effect of biological products on IL-1β production after activation of LPS cells of bacterial origin, were TLR receptor stimulator [46]. Figure 8 shows that the addition of LPS induced the secretion of IL-1β by monocyty macrophage cells in human peripheral blood. The addition of biological products significantly enhanced the response of cells to LPS. Cell cultivation in a medium supplemented with biologics without LPS did not change the level of IL-1β production compared to the control (data not shown), which suggests that horse placenta-based biologics themselves do not have pro-inflammatory properties, but are able to activate IL-1β production by blood monocytes after contact with antigens of bacterial origin.

The significance of differences is presented as * p <0.05 compared to K, + p <0.05 compared to K + LPS. K - cells cultured in PCS without the addition of drugs and LPS; K + LPS - cells cultured in PCB with the addition of LPS (200 μg / ml).

Figure 8 - The effect of biologics based on equine placenta on the production of human IL-1β PBMC. PBMCs were cultured in the presence of various concentrations of bio preparations based on horse placenta for 24 hours, the supernatant was collected and the content of IL-1β was analyzed by enzyme immunoassay. The figure shows the generalized data, n = 4. In those cases, when, when the enzyme-linked immunosorbent assay was performed, the optical density of the previously diluted sample (x20) exceeded the optical density of the calibration curve, the maximum concentration of the calibration curve was multiplied by the dilution coefficient and the obtained value was used.

The discussion of the results

Previously, the ability of some bioactive drugs to restore MTT without the participation of living cells has been shown, which is most often associated with high antioxidant activity or the presence of other non-mitochondrial enzymes [29-31]. So, Chakrabarti et al. showed that ascorbic acid reduces MTT to formazan, while retinol catalyzes this reaction, and the oxidation of ascorbic acid with hydrogen peroxide destroys its ability to restore MTT [30]. The ability to restore MTT has also been shown for the enzyme glutathione S-transferase [31]. Given that horse placenta extracts are a complex mixture of various proteins, cytokines, polypeptides, growth factors, amino acids, lipids, carbohydrates, polyamines, inorganic compounds, hormones and vitamins, we hypothesized that the ability of biological products to intensively restore MTT may indicate their high antioxidant / enzymatic activity. Additional studies are needed to identify the biochemical composition of these biologics. Thus, we concluded that it is impossible to use the standard MTT test to assess the cytotoxicity of biological products.

Cytotoxicity of horse placenta-based biologics with respect to human peripheral blood lymphocytes in the PI inclusion test showed that the biologics did not have a cytotoxic effect on PBMCs after 24 hours of cell culture, regardless of the selected concentration.

Active forms of oxygen (ROS) are produced in oxidation processes and are necessary for energy production to provide other biological processes. However, over-production of ROS damages cells, because ROS are capable of destroying molecules such as DNA and proteins. ROS play an important role in the pathogenesis of
various diseases, such as neurodegenerative, oncological, cardiovascular diseases, atherosclerosis, and others [32]. The mechanism of the acute inflammatory process partially includes the release of ROS from activated neutrophils and macrophages. ROSs spread inflammation by stimulating the release of cytokines such as interleukin-1, tumor necrosis factor-α, and interferon-α, which stimulate the involvement of additional neutrophils and macrophages. Free radicals are important mediators that provoke or support inflammatory processes, and therefore, their neutralization by antioxidants and free radical scavengers can weaken inflammation [33]. Thus, compounds that have the ability to bind these radicals may have therapeutic potential. There are two methods of suppressing ROS. Firstly, natural defense mechanisms that counteract the potential harmful effects of ROS. Cells typically have antioxidant systems that protect against the harmful effects of ROS, including superoxide dismutase (SOD), which converts superoxide anions to hydrogen peroxide (H2O2) for rapid removal using detoxifying enzymes such as glutathione peroxidase. Secondly, the functional components of the environment act as antioxidants [34, 35]. One of these components may be placental extracts [3,4,25]. Thus, it was previously shown that horse placenta extract has a significant cytoprotective effect against skin fibroblast cultures during the induction of oxidative stress using H2O2 in the MTT test [25]. To assess the antioxidant activity of biological products based on horse placenta, we determined the redox status of MNPK by the level of intracellular ROS after 2 hours of incubation in the presence of biological products. Our data are consistent with previously published works on the antioxidant effect of placental extracts shown in various models of oxidative stress [3, 4, 36, 37]. It was suggested that L-tryptophan is one of the main antioxidants of placental extracts [36]. Later in the work of Yamasaki M. et al. it was shown that placental extracts are also able to regulate the activity of genes encoding antioxidant enzymes in cells, such as superoxide dismutase and catalase [37]. It was also noted that the antioxidant activity of placental extracts significantly depends on the conditions for their preparation and storage [38, 39]. The results obtained on the immunomodulation activity of a biological product based on horse placenta for cellular immunity are consistent with a previously published work, which also demonstrated the suppressor activity of an aqueous extract of horse placenta on Con A-stimulated proliferation of murine lymphocytes [25]. The ability of the sheep placenta extract to inhibit the proliferative response of lymphocytes to lectins has also been shown [41]. In another work, it was shown that various fractions of the placental extract can have a stimulating or suppressing effect on mitogen-induced proliferation of lymphocytes [42], which suggests the presence of both immunostimulation and immunosuppressive factors in placental extracts. We can assume that the suppressor effect on mitogen-induced proliferation of T cells by a biological product may be associated with the predominance of immunosuppressive factors such as TGFβ, IL-10 [43] and HLA- G, which provide immunological tolerance to the fetus.

Research data on the possible effect of horse placenta extracts on the cytokine-producing activity of PBMCs have shown the following: Interleukin-4 (IL-4), a short four-helix γ-chain receptor cytokine family polypeptide, is a pleiotropic cytokine produced mainly by Th-2 lymphocytes, basophils and mast cells in response to receptor-mediated activation. IL-4 induces the polarization of CD4+ T cells towards Th-2, while inhibiting IFN-γ-producing Th1 cells. IL-4 supports the growth and differentiation of B-lymphocytes, controlling the specificity of the switching of the class of immunoglobulins G (IgG) and the development of memory B-cells [44]. Thus, our results indicate the ability of biological products. IL-1β is produced by hematopoietic cells such as blood monocytes, tissue macrophages, dendritic skin cells and brain microglia in response to TLR activation, activated complement components, other cytokines (such as TNF-α) and IL-1 itself. IL-1β is an inducible cytokine and is usually not expressed in healthy cells or tissues; however, full-sized IL-1β is rapidly induced in cells after their activation, which leads to intracellular protein accumulation. Processing a full-sized precursor into a biologically active mature protein form requires the presence of caspase-1, which cleaves the N-terminal 116 amino acids from the precursor to form a mature active cytokine. In most cell types, caspase-1 is maintained in an inactive state, and therefore the secretion of active IL-1β is strictly regulated. Inflammation leads to the conversion of pro-caspase-1 into active caspase-1. IL-1β plays a key role in the initiation and regulation of inflammation, activates neutrophils, T- and B-lymphocytes, stimulates the synthesis of acute phase proteins, cytokines (IL-2, IL-3, IL-6, TNF-α), adhesion molecules (E-selectins), prostaglandins, procoagulants, increases chemotaxis, phagocytosis and regulates body temperature [45].

The production and secretion of the pro-inflammatory cytokine IL-1β is crucial for stimulating innate immune responses and attracting phagocytic cells to the site of infection. It was previously shown that biologics with the ability to activate the production of IL-1β can be used to stimulate anti-infection immunity [47]. Thus, the
results indicate the promise of the use of drugs based on horse placenta as a modulator of innate immunity for activation of anti-infection immunity.

Findings:

The conducted studies allow us to draw the following main conclusions:

Biological preparations based on horse placenta do not exert a cytotoxic effect on mononuclear cells of human peripheral blood at 24 hours' exposure at concentrations not exceeding 3250 μg / ml. The bio preparation based on horse placenta extract does not exert a cytotoxic effect in concentrations not exceeding 1500 μg / ml. Biological products have a cytotoxic protective effect against mononuclear cells in human peripheral blood, reducing the intracellular level of reactive oxygen species after 2 hours of exposure. The biological product inhibits mitogen-induced proliferation of T cells in human peripheral blood when used in concentrations non-toxic to cells (375 μg / ml and 1500 μg / ml). Biological products did not induce the secretion of IL-1 by monocytes macrophage cells in human peripheral blood, but they enhanced the response of these cells in response to LPS of bacterial origin, significantly increasing the level of production of IL-1. Biologics induce the production of the anti-inflammatory cytokine IL-4 by mononuclear cells in human peripheral blood. In general, the results demonstrated the potential for further study of the immunomodulation properties of horse placenta extracts and the possibility of their use as immunomodulation drugs with antioxidant properties. It is recommended that additional studies of biological products.

List of used sources


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