The effect of horse placenta extract on human peripheral blood mononuclear cells

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(Received: October 2019 Revised: April 2020 Accepted: May 2020)

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ABSTRACT

Introduction and Aim: Placental extracts due to the unique biological properties have been widely utilized in various fields of medicine. The objective of the study was to investigate the effect of horse placenta extract on human peripheral blood mononuclear cells (PBMCs).

Materials and Methods: The placenta extract was obtained from the mares. PBMCs were isolated from venous blood samples from healthy volunteers. To assess the toxic effect of the placenta extract (in final protein concentrations of 1500, 750, 375, 187.5, 93.75, 46.88 μg/ml) an MTT test was employed. As a control, cultures of PBMCs untreated with an extract were used. The cytotoxicity of the extract was examined by using propidium iodide (PI). A CFSE test was utilized to evaluate T-cell proliferation. The levels of reactive oxygen species (ROS) were also evaluated. An enzyme-linked immune-sorbent assay was used to determine the level of interleukin secretion.

Results: According to the results of MTT assay, the placenta extract cytotoxicity was observed at a concentration of 1500 μg/ml and 750 μg/ml. PI test showed that the placenta extract solution had no toxic effect on PBMCs after 72 h (concentration range from 1500 to 46.9 μg/ml). However, toxic effect was observed after 72 hours of exposure to extract at a concentration of 1500 to 3250 μg/ml, where cytotoxicity was 4.6 and 12.0%, respectively ($p \leq 0.05$). The 2-hour incubation of cells with placenta extract at concentrations of 1500 μg/ml and 375 μg/ml led to a significant decrease in ROS level ($p \leq 0.05$). In addition, T-cell proliferation was considerably reduced at an extract's concentration of 1500 μg/ml and 375 μg/ml compared to the control ($p \leq 0.05$). The detected increase in the production of human IL-4 PBMC (24-hour incubation) was dependent on the concentration of the placenta extract.

Conclusion: The results of the study indicate that the horse placenta extract does not possess cytotoxicity towards human PBMCs. Moreover, we observed the immune-modulating properties of placenta extract.

Keywords: Horse placenta extract; cytotoxicity; immune modulation.

INTRODUCTION

The placenta is an organ responsible for the development and growth of the fetus during gestation (1). In fact, the placenta is the richest source of a range of biological components, such as hormones, cytokines, chemokines, and growth factors (2).

Due to the unique biological properties, placental extracts have been widely utilized in various fields of medicine. To expand the biomedical applications, the effects of human placenta extract have been extensively studied in recent decades. The results of the studies demonstrated the therapeutic potential of placenta extracts in regenerative medicine and wound healing. For example, there is a range of reports indicating the feasibility of using the placental extract in the elimination of menopausal symptoms, without significant side effects (3).

Apart from that, there is accumulating evidence of the curative effect of subcutaneous injections of human placental solution for improving indicators of physical, sexual function and general health (4). Moreover, it was revealed that placenta extracts have anti-aging, anti-oxidative, and anti-inflammatory activities (5). For instance, it was shown that placental extract could play an antioxidant role by stimulating the expression of antioxidant enzyme genes (6).

In an experiment on rats, the anti-oxidative activity of the placental extract in relation to oxidative stress was proved by the decrease in anti-inflammatory cytokines such as TNF-a, IL-1b and IL-6 (7). In another study on the treatment of fatigue syndrome, placental demonstrated a curative effect. The authors concluded that the observed effect can be associated with an increase of antioxidant enzymes and modulation of inflammatory cytokines, which leads to a rise in body energy resources (8).

In another study, topical application of the placental extract showed a therapeutic effect in the treatment of non-healing wounds (9). Due to the presence of growth factors, placental extract demonstrated a positive effect on the acceleration of wound healing.

It was shown that the extract possesses antioxidant and antiapoptotic properties in particular by inhibiting apoptosis through the decrease of the activity of caspase-3 (10).

It is well known that the placenta and fetal membranes are endowed with the immunological properties necessary for a successful pregnancy outcome due to the absence of immunological conflict (11). In fact, pregnancy-related substances and placental hormones can act as immune suppressants. It is important to clarify that the possibility of toxic effects of tissue extracts in vitro. In the experiment, the addition of placenta extract to cultured cells at doses of 200 μg / ml did not cause cytotoxicity; however, further studies are required (12). A recent study showed the ability of a sheep placental extract to suppress over-activation of T-lymphocytes (model of immune-mediated hepatitis) (13). Some researchers pointed out that the inhibitory ability of the placental extract is associated with the proliferative responses of lymphocytes to stimulants with a cytostatic effect (14).

The immuno-regulatory effect of the placenta extract has been employed for the treatment of allergic diseases. In the study conducted by Richard G. Lea the effect of placenta extracts and equine chorionic gonadotropin preparations on lymphocyte proliferation was carried out. It was revealed that the ability of horse placental tissue to suppress the proliferation of lymphocytes was not associated with chorionic gonadotropin (15).

Placental extracts demonstrated the capability of the modulation of the immune system. Moreover, it was shown that the placental extracts can induce secretion of IL-8 in PBMCs via activation of transcription factors and kinase (16).

In our study, the biological activity of the horse placental extract was studied on human PBMCs. In fact, PBMCs are the central unit of the immune defense of the organism (17). These immune cells play a key role in autoimmune responses and the development of several types of chronic inflammatory processes. In addition, PBMCs can be used to assess cellular immunity, detect previous exposure to various antigens and monitor the response to immunotherapy (18).

The presented study is aimed at assessing the cytotoxicity, antioxidative and other biological effects of the horse placental extract on PBMCs.

MATERIALS AND METHODS

Obtaining an extract of the placenta

The horse placenta was obtained from the environmentally friendly farms of the Akmola and Karaganda regions of the Republic of Kazakhstan. The placenta was taken immediately after the vaginal delivery from the mares, and the umbilical cord and amnion were disposed. The preparation of the placenta extract was carried out according to the patent (19), where the remaining tissue was thoroughly washed with ice-cold phosphate-saline buffer to completely remove all traces of blood. Placental tissues were destroyed employing 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. The placenta extract was sterilized using γ-radiation generated by using ILU-10 accelerators and cobalt-60 isotopes (Russia) at the Institute of Nuclear Physics in Almaty, Kazakhstan. The absorbed dose of γ-irradiation was 30 kGy at a speed of 1 kGy / h.

In vitro study of the immune-modulating properties of the horse placenta extract

Venous blood samples

Samples of venous blood were obtained from healthy donor volunteers (an average age 39.6 ± 13.5 years (range 19-72), n = 16; women: an average age 42.3 ± 21.7 years, n = 6; men: an average age 37.9 ± 5.7 years, n = 10). Venous blood was taken from the ulnar vein for 9-18 ml into sterile tubes with EDTA (VenoSafe) with a nominal volume of 9 ml using the Venosafe Quick Fit holder. The study was approved by High Ethical Committee of JSC "National Medical University" (protocol No. 7 (84) by June 10, 2019). All donors provided and signed the informed consent. The study was conducted in accordance with the ethical principles of the Helsinki Declaration.

Determination of protein concentration in horse placenta extract.

The determination of the protein concentration in the sample of the placenta extract was determined by measuring the optical density of the solution (absorption) at 280 nm and 260 nm wavelength (spectrophotometer). The calculation of protein concentration in solution was carried out according to the previously described method (20).

Isolation of the mononuclear fraction of cells from peripheral blood

To isolate peripheral blood mononuclear cells (PBMCs), 10 ml of whole blood was layered on 10 ml of histopack-1077 or percoll of an appropriate density, and centrifuged for 30 min at 400 g at 20°C. Interphase cells of the mononuclear ring were washed 2 times with a 20-fold volume of RPMI-1640 medium at 200 g for 15 min at 20°C.

To assess the toxic effect of horse placenta extract, an MTT test was used

For this, PBMCs were cultured in 96-well plates at a concentration of 1 × 1x105 / well in 200 μl of PBS for 24 hours at 37°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 complete cell culture medium. At the end of the incubation, 20 μl of MTT solution (5 mg/ml) was added to each well and further incubated for 4 hours at 37°C. Then the supernatant was removed, and the precipitate was dried at room temperature, and the resulting formazan crystals were dissolved in 100 μl of DMSO. The optical density was measured on an immunoassay analyzer at 492/630 nm. The cytotoxicity index was determined according to the standard procedure.

Assessment of cytotoxicity by the inclusion of propidium iodide (PI)

Horse placenta-based extract cytotoxicity was evaluated by using propidium iodide (PI). PBMCs were cultured in 10% PBS at a concentration of 5x105 / ml in the presence of various concentrations of the biological product in a final volume of 1 ml for 24 and 72 hours at 37 ° C and 5% CO_2. Then, the cells were then washed with PBS at 300 g for 5 min and re-suspended in flow cytofluorimetry buffer. PI was added according to the manufacturer's instructions, incubated for 1 min in the dark, and then analyzed on a FACSCalibur flow cytometer (BD Biosciences, USA) using FL-2. Data analysis was performed using CellQuest Pro software (BD Biosciences, USA). Unpainted cells were used as control. As minimal quantity for analysis, at least 50 thousand cells were used. Lymphocytes and monocytes were gated on the FSC vs SSC Dot plot, 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 cell death.

CFSE T cell proliferation assay

Carboxy fluorescein succinimidyl ester (CFSE) is a non-fluorescent label. Under the influence of intracellular esterases in living cells, CFSE transforms into a fluorescent molecule that reacts with intracellular free amines to form covalent stable dye-protein conjugates. The protein-dye conjugate, which forms in labeled cells, is retained by these cells for several dividing cycles. Therefore, CFSE is used to evaluate cell proliferation.

To assess the effect of the biological product on the proliferation of T cells, PBMCs (obtained from volunteer donors) were re-suspended in RPMI-1640. Then, a CFSE solution (5μM) was added and incubated for 6 min at 4°C. After this, the cells were washed twice with cold PBS. Labeled CFSE cells were cultured in PBS in the presence of 5 μg/ml ConA at 37°C and 5% CO_2 at a final concentration of 2 × 106 cells/ml. As a control, control samples were used without adding 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.

ROS product evaluation

The antioxidant activity of the biological product was evaluated by using a DCFDA (2′-7′-Dichlorodihydrofluorescein diacetate) and 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' dichloro-fluorescein (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. A DCFDA stock solution (5 mM) was prepared in DMSO and stored at -20°C. The effect of biologics on the production of human PBMCs was studied according to the previously described methodology (21).

Briefly, the resulting PBMCs were re-suspended in 10% PBS at a concentration of 5 × 105 cells/ml and incubated for 24 hours at 37°C and 5% CO_2. Next, the cells were washed with PBS, pre-treated with 2 μM DCFDA in a 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 products. Then the cells were washed and immediately analyzed on a flow cytometer.

ELISA

The effect of biological products on the level of secretion of IL-4, IL-4 PBMCs was analyzed using ELISA using commercial kits ("Vector-Best"). The level of IL-4 PBMC secretion in the culture supernatants was evaluated after 24-hour LPS cell stimulation (200 mg/ml).

Statistical analysis

Statistical analysis was performed by using SPSS V22.0 (USA) software. Flow cytometry data was calculated and analyzed by using Flowing program 2.5.1. (Finland). All analyzes were performed at least 10 times. The information is presented in the form of an average 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. Differences were considered insignificant if the probability of the null hypothesis did not exceed 5% ($p< 0.05$).

RESULTS

During the preparation of the horse placenta extract, we obtained a substance in the form of a powder free

of foreign particles (color: from white to slightly yellow). Adding the solvent resulted in the formation of a solution of a translucent liquid with visible particles, light yellow in color.

The protein concentration in the resulting solution after a 3-fold measurement averaged 5.34 ± 0.9 mg / ml. The cytotoxic effect of horse placenta extract in the MTT test was studied at protein concentrations of 1500, 750, 375, 187.5, 93.8, and 46.9 µg/ml (Fig. 1). As a control were used cells incubated in 10% PBS (intact cells).

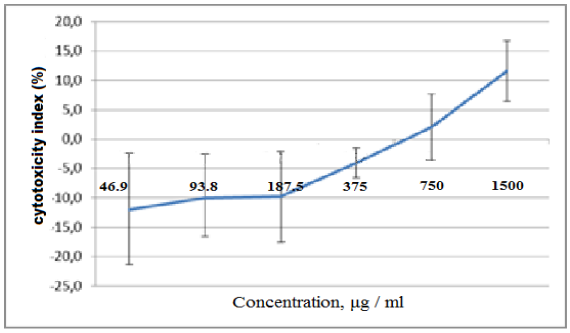

Fig. 1: Cytotoxic effect of horse placenta extract in MTT assay at protein concentrations of 1500, 750, 375, 187.5, 93.8, 46.9 µg/ ml

The results of MTT test showed that the cytotoxicity of the placenta extract regarding PBMCs was observed at a concentration of 1500 µg / ml and 750 µg / ml that corresponds to a cytotoxicity index of 12 ± 4.4% and 2.6 ± 7.9%, respectively.

However, at concentrations of 375, 187.5, 93.8, and 46.9 µg/ml, placenta extracts stimulated cell proliferation and metabolic activity (compared with the control ($p \leq 0.05$)).

Assessment of cytotoxic activity by using a test with the inclusion of propidium iodide (PI) showed that the placenta extract solution had no toxic effect on human PMBCs after 72 hours when it was added in a concentration range from 1500 to 46.9 µg / ml (Fig. 2). However, we also observed induced cell death ($p \leq 0.05$) compared with the control after 72 hours of exposure at a concentration of 1500 to 3250 µg / ml, where cytotoxicity was 4.6 and 12.0%, respectively.

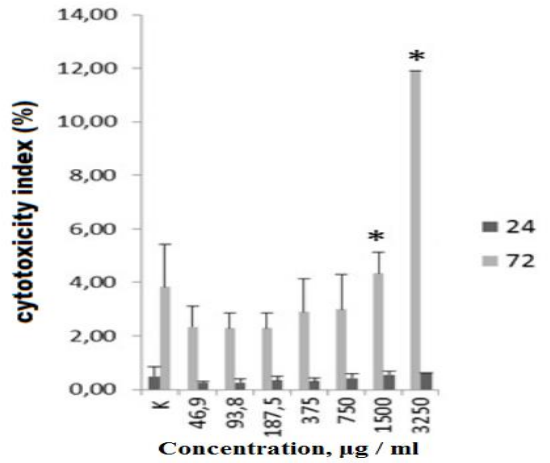

Fig. 2: The results of cytotoxicity test (PI inclusion)

Note: The significance of differences is presented as *p <0.05 compared to corresponding control. K - cells cultured in PBS without the addition of drugs, stained with PI.

A study of the antioxidant activity of horse placenta extract showed that 2-hour incubation in the presence of the extract at concentrations of 1,500 µg / ml and 375 µg / ml significantly reduced the ROS level ($p \leq 0.05$) in human PBMCs compared to cells incubated in PBS without extract (control).

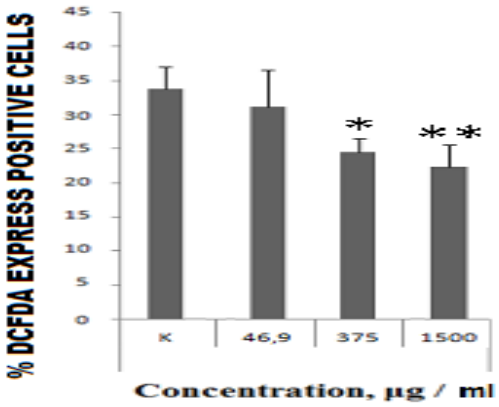

Fig. 3: Production of ROS human PBMCs after 2 hours of incubation with horse placenta extract, (M ± SD)

Note: The significance of differences is presented as *p <0.05 and **p <0.005 compared to the control.

In turn, the effect of horse placenta extract on mitogen-stimulated T-cell proliferation in vitro (Fig. 4) showed that the extract significantly inhibited T-cell proliferation at concentrations of 1500 µg/ml and 375 µg / ml compared to the control ($p \leq 0.05$).

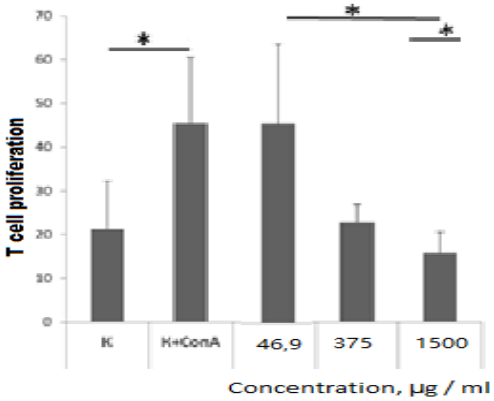

Fig. 4: Effect of horse placenta extract on mitogen-stimulated T-cell proliferation in vitro (M ± SD)

Note: K - cells cultured in PKC without the addition of drugs stained with CFSE; K + ConA - cells cultured in PBS with ConA (5 µg/ml) without the addition of preparations stained with CFSE.

The results of a study of the effect of horse placenta extract on IL-4 production (Fig. 5) showed that, depending on the concentration of the extract solution, there is an increase in the production of human IL-4 PBMCs compared to the control during 24-hour incubation.

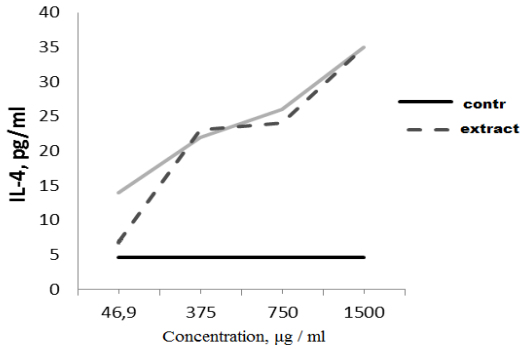

Fig. 5: Effect of placenta extract on human IL-4 PBMC production during 24-hour incubation.

A study of the effect of placenta extract on the production IL-1β showed a significant increase in cell response to LPS. The cultivation of cells in a medium did not change the level of IL-1β production as compared to the control that suggests that the biologics extract based on horse placenta does not have anti-inflammatory properties. However, the results indicate that the placenta extract is able to activate the production of IL-1β by blood monocytes after contact with antigens of bacterial origin.

DISCUSSION

Up to date, a range of studies on the bio-effects of placenta extracts have been conducted (2). It encompasses the research focused on the extracts obtained from human or animals placental tissues, such as sheep, goats, cattle and horses (16,19,24,25).

The standard placental extracts do not contain cells, but have a wide range of proteins, minerals, amino acids and steroid hormones. Nowadays, various animal placenta extracts have been widely used for the production of pharmaceuticals (4). It has been shown that the application of animal extracts can improve metabolism at the cellular and tissue levels (10). Particularly, there is a growing body of evidence that the placenta extracts possess restorative, immuno-modulating and anti-inflammatory properties (16).

It has been also shown that placenta-based drugs favor reparative processes in the organism (9). These findings spurred the research on the impact of placenta extracts on the regeneration and immune system. At present, different methods for the production of placenta-based extracts have been proposed. However, the current methods possess various flaws such as instability of compounds and low therapeutic effects.

In the presented study the sterilization of the placenta extract was carried out using γ-radiation, where the absorbed dose of γ-radiation was 30 kGy at a rate of 1 kGy / h. Such a method allows the prevention of the denaturation of protein components, and the formation of a translucent a solution with visible particles (color: light yellow).

The results of cytotoxicity studies (MTT assay) demonstrated that the addition of MTT after 24 hours of cell incubation led to a very rapid formation of formazan crystals (regardless of the presence of cells). This effect can be explained by the ability of the placenta extract to restore MTT. We observed a traceable cytotoxicity of the placenta extract at a concentration of 1500 µg/ml and 750 µg/ml.

At the next stage of the work, in order to assess the cytotoxic activity of the horse placenta extract, we used a propidium iodide (PI) test. PI is a membrane-affinitive dye that does not stain living cells, but penetrates through the damaged membranes of dead and apoptotic cells, and then, binds to their double-stranded DNA. Special attention was paid to the preservation of 10% FBS concentration. The results of the study showed that the horse placenta extract did not exert a cytotoxic effect towards PBMCs after 24 hours of incubation, regardless of the chosen concentration.

The discrepancy in the results of the MTT test and the PI test can be explained by the fact that the MTT test is an indicator of the metabolic activity of cells. So that MTT data are highly susceptible to metabolic interference that can lead to false-positive results. In this regard, metabolism-based tests are considered useful for obtaining preliminary information, but cannot be used for the identification of the cell death, and distinguishing between cytotoxic and anti-proliferative effects.

The results obtained allow us to hypothesize that the horse placenta extract (even after prolonged cultivation) does not possess a cytotoxic effect towards human PBMCs in concentrations not exceeding 1500 µg / ml. However, the increase of concentration higher than 1500 µg/ml led to the fall of cell viability. The observed cell toxicity can be explained by the increase of concentration of placenta ingredients, such as steroid hormones and other compounds. In fact, placental extracts are rich in various types of hormones, vitamins and biologically active substances. However, further intensive studies on the effect of placental extract concentrations on cell viability are required.

To assess the antioxidant activity of biological products based on horse placenta, we determined the redox status of PBMCs by the level of intracellular Reactive Oxygen Species (ROS) after 2 hours of incubation. The results showed that a 2-hour incubation in the presence of a placenta extract at concentrations of 1500 µg/ml and 375 µg/ml significantly reduced the ROS level in human PBMCs compared to the sham.

ROS are produced during the oxidation processes. ROS are necessary for energy production necessary for other vital biological processes. However, over-production of ROS damages cells, because ROS are

capable of destroying molecules such as DNA and proteins. In fact, ROS play an important role in the pathogenesis of various diseases, such as neurodegenerative, oncological, cardiovascular diseases, atherosclerosis and others (22). The mechanism of the acute inflammatory process partially includes the release of ROS from activated neutrophils and macrophages. ROS 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 (23). 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. Typically, cells have antioxidant systems that protect against the harmful effects of ROS, including superoxide dismutase (SOD), which converts superoxide anions to hydrogen peroxide (H_2O_2) for rapid removal using detoxifying enzymes such as glutathione peroxidase. Secondly, the functional components of the environment act as antioxidants. One of these components may be placental extracts. Thus, it was previously shown that horse placenta extract has a significant cyto-protective effect against skin fibroblast cultures during the induction of oxidative stress using H_2O_2 in the MTT test (24).

To the best of our knowledge, this is the first study on the effect of placental extract on the production of intracellular ROS (in human PMBCs). Our data are consistent with previously published reports on the antioxidant effect of placental extracts, shown in various models of oxidative stress (6, 7). The antioxidant property of the placental extracts can be explained by the presence and activity different components of the extracts. For instance, it has been suggested that L-tryptophan is one of the main antioxidants of placental extracts. Yamasaki *et al*., demonstrated that placental extracts are also able to regulate the activity of genes encoding antioxidant enzymes in cells, such as superoxide dismutase and catalase (6). It was also showed that the antioxidant activity of placental extracts mainly depends on the conditions for their preparation and storage.

To assess the immune-modulating activity of extracts based on horse placenta for cellular immunity, we evaluated the mitogen-induced blastogenic response of PBMCs during cultivation. Conconavalin A (Con A), a widely used antigen-independent mitogen that binds mannose residues of cell surface glycoproteins, including the T-cell receptor, and leading to polyclonal proliferation of T cells, was used as a mitogen (25). The results are consistent with previously published reports that also demonstrated the suppressor activity of an aqueous extract of horse placenta on Con A-stimulated proliferation of murine lymphocytes. The ability of the sheep placenta extract to inhibit the proliferative response of lymphocytes to lectins has been also previously shown. In another study, it was demonstrated that various fractions of the placental extract might have a stimulating or suppressing effects on mitogen-induced proliferation of lymphocytes. These findings indicate the presence of both immune-stimulatory and immune-suppressive factors in placental extracts. Therefore, we hypothesize that the suppressor effect exerted on mitogen-induced proliferation of T cells by the placental extract may be associated with the predominance of immunosuppressive factors such as TGFb, IL-10 (26) and HLA-G, which provide immunological tolerance to the fetus.

Interleukin-4 (IL-4), a short four-helix γ-chain receptor family cytokine polypeptide, is a pleiotropic cytokine produced primarily by Th-2 type 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 (27). Thus, our results indicate the ability of placenta extract to modulate the Th-2 immune response through the induction of production of IL-4.

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 procaspase-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 (28).

A study of the effect of placenta extracts on IL-1β production showed that the addition of LPS induced the secretion of IL-1β by monocytic macrophage cells in human peripheral blood. The addition of horse placenta extract significantly enhanced cell response to LPS. The cultivation of cells in a medium with the addition of extracts without LPS did not change the level of production of IL-1β compared with the control, which suggests that the horse placenta extract does not have a pro-inflammatory property, but it is able to activate the production of IL-1β by blood monocytes after contact with bacterial antigens origin.

The production and secretion of the anti-inflammatory cytokine IL-1β are crucial for stimulating innate immune responses and attracting phagocytic cells to the site of infection. It was previously shown that biological products with the ability to activate the production of IL-1β can be used to stimulate anti-infection immunity (29). Thus, the results of presented study indicate the promise of using an extract based on horse placenta as a modulator of innate immunity for activation of anti-infection immunity.

CONCLUSION

The results of our study demonstrated the therapeutic potential of an extract based on horse placenta as a modulator of innate immunity for the activation of immune defense of the organism. However, further intensive studies are necessary in order to validate the clinical relevance and therapeutic efficacy.

ACKNOWLEDGMENTS

The authors are thankful for the support through the grant: No. AR05134106 of the Ministry of Education and Science of the Republic of Kazakhstan "Development of biologically active preparations based on horse placenta"

CONFLICT OF INTEREST

The authors declare that they have no competing interests related to this work.

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