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INFLUENCE OF PEPTIDES FROM THE BURSA OF FABRICIUS IN BROILER CHICKENS ON THE FUNCTIONAL ACTIVITY OF LYMPHOCYTE SUBPOPULATIONS IN IMMUNODEPRESSIVE MICE

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Abstract
It is known that peptides inhibit the enzymes of viruses and are able to penetrate into cells by their embedding in the cell membrane, as a result of which the penetration of viruses into the host cell is blocked, which makes it possible to consider peptides as an alternative to antiviral drugs. In this regard, the demand for immune-boosting nutraceuticals and functional foods containing biologically active peptides is growing. The immunomodulating effect of the peptides were studied on the mice of the BALb/c line that suffered from experimentally induced immunodeficiency; the mice got injections of peptides isolated from the bursa of Fabricius (bursal sac) of broiler chickens. 5 groups of BALb/c mice were formed. The animals of the 1st group (control one) received physiological saline per os as a placebo, animals of the 2nd group got bursal peptides per os at a dose of 0.02 mg/kg per body weight, the mice of 3rd group (immunosuppressed) got saline per os as a placebo, the 4th group (immunosuppressed) was administered the bursal peptides per os at a dose of 0.02 mg/kg of body weight, the 5th group was held as the control one (immunosuppressed group). Blood for tests was taken on days 1, 7 and 14 of the experiment. The functional activity of neutrophils was determined by the method of spontaneous and induced chemiluminescence. Among the immudepressive animals (the 3rd group) on the 7th day the researchers observed a decrease in CD3+ by 55.3%, CD22+ by 83.7%, CD3+CD4+ by 51.9% and CD3+CD8+ by 54.6% in comparison with the intact (the 1st group). Administration of peptides to immunosuppressed mice (the 4th group) increases the number of subpopulations of CD3+ lymphocytes by 126.6%, CD22+ by 381.6%, CD3+CD4+ by 8.9% and CD3+CD8+ by 81.8% compared to immunosuppressed animals, receiving saline per os as a placebo (group 3). Similar results were obtained on the 14th day of the experiment. On the basis of the performed studies, it can be argued that the immunocompetent organs of broiler chickens (bursa of Fabricius) are a promising source of immunotropic peptides.).

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Introduction

Biologically active peptides (BAP) are considered to be the main products of protein hydrolysis. The activity of BAP depends on the sequence of amino acid, pf molecular weight and chain length, type and charge of the amino acid at the N-terminus and C-terminus, hydrophobicity and hydrophilicity of their spatial structure. They provide a positive effect on many systems of the human body, including the blood circulatory system, nervous, immune, gastrointestinal system and others. According to [1], the curative effect of bioactive peptides is achieved due to their antioxidant, antihypertensive, antithrombotic, immunomodulatory, antimicrobial, antiallergic, anti-inflammatory and other propeties.

Currently there is a growing demand for immune-boosting nutraceuticals and functional foods that contain immunomodulatory proteins of animal origin, in particular, BAP [2].

However, it is necessary to take into account the lack of correlation between the results obtained *in vitro* and the peptides functions observed *in vivo* due to their low bioavailability. Once ingested, the peptides must resist the action of digestive enzymes during their passage through the gastrointestinal tract and have to cross the intestinal epithelial barrier to reach target organs in an intact and active form. Thus, in order to understand better the physiological effects of bioactive peptides *in vivo*, more extensive studies of their stability and transport in the gastrointestinal tract, as well as the study of the mechanism of action, turned out to be necessary [3].

Peptides act as signal substances containing fundamental molecular information, peptides are able to penetrate cell membranes or reach intracellular targets [4].

The immunomodulatory effect of peptides allows them to be involved in cancer therapy. However, cancer immunotherapy so far could not improve the outcomes for most cases of "cold tumors", which tumors are characterized by low immune cell infiltration and an inner immunosuppressive tumor microenvironment. Increasing the sensitivity

of cold tumors to cancer immunotherapy by stimulating components of the tumor microenvironment is a strategy being pursued in the last decade. Currently, most of the drugs, used to modify the tumor microenvironment are the small molecules or antibodies. Small molecules exhibit low affinity and specificity in relation to the target tissue, and antibodies have certain disadvantages such as poor tissue penetration and high cost of production. Peptides do not have those disadvantages and, therefore, can serve as a promising material for immunomodulatory agents [5].

Particular attention should be given to immunomodulators based on peptides isolated from meat raw materials, tissues of immunocompetent organs of animals and poultry, in particular, glucosaminylmuramyl dipeptide (GMDP), registered under the name Likopid® [6], and also obtained from biological fluids, for example, from cow colostrum.

Biologically active peptides are found in raw meat, and for their greater accumulation the starter cultures of microorganisms and proteolytic enzymes are used. Using T-RFLP analysis, it was found that dry-cured and raw-smoked sausages made from horse meat using starter cultures contain short peptides of horse myoglobin, troponin-T, and muscle creatine kinase [7].

The immunocompetent organ in a chicken is the bursa of Fabricius (bursa of Fabricius, sac of Fabricius, or bursa), where plasma cells are formed, that synthesize antibodies. Removal of the bursa in poultry leads to inhibition of antibody biosynthesis. 20 peptides with immunomodulating properties have been isolated from the bursa [8]. The peptides isolated from the bursa inhibit the growth of cancer cells by discontinuance of the cell life cycle in the G1 phase, i. e. the peptides stop the process of formation of a new structure formed by the centriole of the mother cell — the primary cilium, which protrudes from the cell surface — this process is not initiated [9].

The source of peptides possessing antiviral and antitumor properties is cow colostrum, in particular, proline — this is polypeptide (PRP), or a transfer factor — a stimulator of lymphocytes (T-killers) proliferation, which cells feature cytotoxicity against tumor cells and virus infected cells. The daily intake of dry colostrum by healthy people for two weeks at a dose of 400 mg stimulates humoral and cellular immunity [10].

The aim of the research was to study the effect of immunotropic peptides isolated from the broiler chicken bursa of Fabricius on the decrease in the functional activity of lymphocyte subpopulations.

Materials and methods

For the experiment peptides were isolated from the broiler chicken bursa of Fabricius (Pervouralskaya poultry farm) using the following technology: the raw material was washed with running water, ground in a laboratory mill (OLIS, Russia), and a solution was prepared with a hydromodulus 1:3 (crushed bursa of Fabricius: distilled water), papain enzyme was introduced (Enzyme BioScience (P) Ltd, India) (0.15% from the weight of the raw material) in a phosphate buffer solution prepared by us (disodium hydrogen phosphate dodecahydrate (Rosspolymer, Russia), citric acid monohydrate (Alkhimpro, Russia) at pH 6; the obtaid mixture was exposed to hydrolysis for 12 hours at a temperature of 36 °C; after that the temperature was increased up to 75 °C to inactivate the enzyme. Moisture was removed from the enzymatic hydrolyzate of the bursa of Fabricius by freeze drying (Institute of Biological Instrumentation, Russian Academy of Sciences, Russia) at a temperature of minus 40 °C till achieving moisture content of 6%.

The molecular weight distribution of the peptides was implemented by mass spectrometry and was identified by MALDI-TOF and MS/MS mass spectrometry on the device Ultraflex MALDI time-of-flight mass spectrometer (Bruker, Germany). Mass spectra analysis was run with the help of Mascot software, Peptide Fingerprint option (Matrix Science, USA), using the Protein NCBI database.

The peptides effects on the immune system were studied on BALb/c mice with induced experimental immunodeficiency. In order to induce the immunosuppression, the day before the experiment the exposed animals got one intraperitoneal injection with the cytostatic Cyclophosphan (Lens-Pharm, Russia) at a dose of 100 mg/kg. The number of surviving animals was equal to 100%. Usually three doses every 72 hours are given. The immunomodulatory effects were studies on the examples of the mice that got *per os* the peptides isolated from the bursa of Fabricius of broiler chickens. The BALb/c mice were sorted into 5 groups, including 3 groups of mice with experimental immunodeficiency. Animals of the first (control) group received saline *per os* as a placebo, animals of the second group received bursal peptides *per os* at a dose of 0.02 mg/kg of body weight for 7 days (the amount of peptides was determined by the protein content by the Keldahl method on the automatic protein analyzer (ERKAYA, Turkey), the third group (immunosuppressed) got saline solution *per os* as a placebo, the fourth group (immunosuppressed) was injected with bursal peptides, the fifth control group was a control group (immunosuppressed mice). The amount of peptides for feeding the laboratory animals was calculated according to the recommendations [11].

On the 1st, 7th, and 14th days of the experiment, blood was taken into BD Microtainer® tubes with EDTA anticoagulant (Beckman Coulter, USA) by cutting off a 1 mm tail tip. Blood samples from experimental and control groups of mice were stained with monoclonal antibodies CD22-PE-CY5, CD3-FITC, CD4-APC, CD8-PE (BDPharmingen™, USA) according to the manufacturer's instructions. For blood staining an appropriate volume of fluorochrome-conjugated monoclonal antibodies was added to 100 µl of whole blood in a 12 x 75 mm polystyrene tube with a cap closure; the contents of the test tubes were shaken and incubated for 20 minutes in the dark at a tem-

perature of 23–25 °C; 2 ml of non-concentrated BD FACS lysing solution (Becton, Dickinson and Company BD Biosciences, USA) was added to each tube and the contents of the tubes were shaken, incubated for 10 minutes in the dark at room temperature; centrifuged (ELMI, Latvia) for 5 minutes at 3,000 rpm. The supernatant was taken and resuspended in a buffer solution (Becton, Dickinson and Company BD Biosciences, USA); 0.5 ml of fixing agent Perfix-ne was added (Beckman Coulter Life Sciences, USA); the contents of the tube were mixed, incubated at 6–8 °C for 30 minutes. The obtained substance was studied 2 hours after staining of blood cells. To calculate the absolute number of lymphocytes in the blood, BD Trucount Tubes cytometric tubes (Becton Dickinson and Company, USA) were used. The number of lymphocyte populations with surface markers was determined on a FACSCalibur cytofluorimeter (Beckton and Dickenson, USA) using the CellQuestPro software. The effect of drugs on the functional state of lymphocytes was assessed by changing the number of lymphocytes populations CD3+ (T-lymphocytes), CD22+ (mature B-lymphocytes), CD3+CD4+ (T-helpers), CD3+CD8+ (cytotoxic T-lymphocytes) in the whole blood samples [12].

The functional activity of neutrophils was determined by the method of spontaneous and induced chemiluminescence (CL). As objects of study neutrophils of the peritoneal exudate of mice were chosen. To obtain peritoneal exudate, animals were euthanized using cervical dislocation. The surgical area was treated with a 70% ethanol solution, then 10 ml of the RPMI-1640 nutrient medium (M. P. Chumakov Plant for bacterial and viral preparations production, Russia) was injected into the abdominal cavity, massaged, and in 5 minutes the abdominal cavity was opened, 10 ml of liquid was taken with a syringe and the liquid centrifuged for 10 minutes at 3,000 rpm, the supernatant was drained off.

To activate phagocytes the opsonized zymosan (manufactured by Merck KGaA, Germany) was used as phagocytosis inducer. Neutrophils of the peritoneal exudate of intact mice (group 1) were taken as a control reference according to the above-described method.

During the experiments the phagocytes suspension was added to each well of a 96-well plate for scintillation counting. so that the final concentration was 0.5x106 cells/ml, which was counted in a Goryaev counting chamber, then a suspension of zymosan was added at a concentration of 2 mg/ml, 0.02 ml per each well and 5.6 × 10–4 96% solution of luminol was added (Panreac / AppliChem, USA) in amount of 0.02 ml each well. The plates were placed in a universal thermostatted plate scanner Victor for detection of fluorescent signals (manufactured by PerkinElmer, USA) and chemiluminescence was measured at 37 °C at 0.1-minute intervals for 100 minutes long. The chemiluminescence level was assessed by kinetic curve change in the registered pulses, recorded by the Victor fluorescent signal scanner hardware-software complex (manufactured by PerkinElmer, USA). The device registered the signal amplitude at its maximum and registered the light sum.

The functional activity of phagocytes was analyzed on the basis of assessment of chemiluminescent activity of peritoneal neutrophils in healthy and in immunodeficient mice at different times after the completion of the course of taking the peptides. The intensity of the chemiluminescent response was estimated from the maximum value of neutrophil impulses on the kinetic curve. The mean chemiluminescence intensity was determined from three identical measurements using a Victor fluorescent signal scanner. The results were evaluated by the number of pulses for minute per 1 μl of peritoneal exudate per the number of neutrophils in 1 μl.

Data was analyzed in the STATISTICA 9.0 statistical software package. Data are presented as arithmetic mean (M) + standard error of the mean (m). To test the hypothesis about the homogeneity of two independent samples, the nonparametric Mann-Whitney Utest was used. The discrepancies between the obtained values were considered significant when $p < 0,05$.

Results and discussion

We have conducted studies on the study of the immunomodulatory effect of peptides isolated from the bursa of Fabricius in broiler chickens. The absolute content of lymphocytes subpopulations in intact mice blood is presented below in the Table 1.

Among the immunosuppressed mice at various times of the study there was a decrease in number of analyzed lymphocytes subpopulations (CD3+, CD22+, CD3+CD8+) in comparison with the intact group ($P \leq 0.05$). Among the animals that were injected with CF (the 5th group) on the 7th day there was a decrease in CD3+ by 55.3%, CD22+ by 83.7%, CD3+CD4+ by 51.9% and CD3+CD8+ by 54.6% in relation to the intact (the 1st group). The introduction of bursal peptides to the animals (the 2nd group) contributed to a significant ($P \leq 0.05$) increase from 0.637 to 1.044 of cells number per liter, ×109 on the 7th day CD22+ or by 61.0% in relation to the intact (the 1st group). When peptides were used in immunosuppressed mice (the 4th group) there was an increase in subpopulations of CD3+ lymphocytes from 0.873 to 1.976 cells per liter, ×109 or by 126.4%, CD22 from 0.112 to 0.429 cells per liter, ×109 or by 283.0 .6%, CD3+CD4+ from 0.936 to 1.326 cells per liter, ×109 or 41.7% and CD3+CD8+ from 0.175 to 0.318 cells per liter, ×109 or 81.7% in comparison with immunosuppressed animals treated *per os* with saline as a placebo (group 3) ($P \leq 0.05$).

Similar results were obtained on the 14th day of the experiment. Animals treated with cyclophosphan (group 5) showed a decrease in CD3+ by 60.0%, CD22+ by 26.1%, CD3+CD4+ by 32.7% and CD3+CD8+ by 22.6% in relation to the intact group (the 1st group) ($P \leq 0.05$). The inclusion of bursal peptides in the diet of animals (the 2nd group) significantly increased ($P \leq 0.05$) the number of

Table 1. Absolute content of lymphocyte subpopulations in whole blood of mice in the control and the experimental groups

Number of cells per one liter, ×10⁹				
1st day				
Group	CD3 + (T-lymphocytes)	CD22 + (mature B-lymphocytes)	CD3 + CD4 + (T-helpers)	CD3 + CD8 + (cytotoxic T-lymphocytes)
1 (intact group / control)	2.413 ± 0.045	0.659 ± 0.021	1.723 ± 0.031	0.471 ± 0.031
2 (bursal peptides)	2.203 ± 0.034*	0.632 ± 0.013*	1.727 ± 0.032	0.394 ± 0.037*
3 (immunosuppressed + per os saline as placebo)	1.292 ± 0.023	0.130 ± 0.0282	1.057 ± 0.024	0.199 ± 0.031
4 (immunosuppressed + bursal peptides)	1.432 ± 0.035**	0.437 ± 0.026**	1.521 ± 0.027**	0.342 ± 0.026**
5 (Cyclophosphan)	1.294 ± 0.027***	0.128 ± 0.022***	1.052 ± 0.032***	0.196 ± 0.019***
7th day				
1 (intact group / control)	2.425 ± 0.042	0.637 ± 0.024	1.618 ± 0.042	0.467 ± 0.033
2 (bursal peptides)	2.034 ± 0.031	1.044 ± 0.027*	1.559 ± 0.030	0.453 ± 0.039
3 (immunosuppressed + per os saline as placebo)	0.873 ± 0.025	0.112 ± 0.027	0.936 ± 0.026	0.175 ± 0.021
4 (immunosuppressed + bursal peptides)	1.976 ± 0.025**	0.429 ± 0.029**	1.326 ± 0.026**	0.318 ± 0.031**
5 (Cyclophosphan)	1.085 ± 0.025***	0.104 ± 0.024***	0.778 ± 0.022***	0.212 ± 0.025***
14th day				
1 (intact group / control)	2.398 ± 0.037	0.639 ± 0.021	1.625 ± 0.035	0.452 ± 0.039
2 (bursal peptides)	2.244 ± 0.036	1.051 ± 0.034*	1.722 ± 0.036	0.366 ± 0.034
3 (immunosuppressed + per os saline as placebo)	0.827 ± 0.022	0.098 ± 0.025	0.875 ± 0.023	0.307 ± 0.028
4 (immunosuppressed + bursal peptides)	1.521 ± 0.027**	0.426 ± 0.024**	0.953 ± 0.028**	0.296 ± 0.025
5 (Cyclophosphan)	1.056 ± 0.024***	0.472 ± 0.029***	1.093 ± 0.023***	0.350 ± 0.028***

* — differences from the 1st group of animals (intact control group) are significant (P ≤ 0.05);
** — differences from the 3rd group of animals (immunosuppressed + per os saline as placebo) are significant (P ≤ 0.05);
*** — differences with the 1st group 1 of animals (control) are significant (P ≤ 0.05)

subpopulations of CD22+ lymphocytes from 0.827 to 1.521 cells per liter, ×109 or by 83.9%, in relation to the intact (the 1st group) (P ≤ 0.05). It was shown administration of bursal peptides to immunosuppressed mice (the 4th group) on 14th day leads to an increase in subpopulations of CD3+ lymphocytes from 0.827 to 1.521 cells per liter, ×109 or by 83.3%, CD22+ from 0.098 to 0.426 cells per liter, ×109 or by 334.7% and CD3+CD4+ from 0.875 to 0.923 cells per liter, ×109 or by 5.5% compared with immunosuppressed animals treated *per os* with saline as placebo (group 3) (P ≤ 0.05).

Figure 1 shows the data of induced chemiluminescence of mice neutrophils on the 1st day of the experiment.

From the presented kinetics of neutrophils chemiluminescence it follows that under the influence of the immunosuppressor cyclophosphan, there is a slight decrease in the level of induced CL (the 5th group). The highest level of CL is observed when it was exposed to bursal peptides (the 2nd group) — it was significantly higher than the control group by 112 imp/sec (P ≤ 0.05) or 108% (P ≤ 0.05) at the 70th minute. Under the influence of bursal peptides in immunosuppressed mice (the 4th group) there is a well pronounced increase in chemiluminescence — an increase in the level of CL in comparison with the 3rd group (immunosuppressed + *per os* saline as a placebo) by 90 imp/sec or 84.4% (P ≤ 0.001) and by

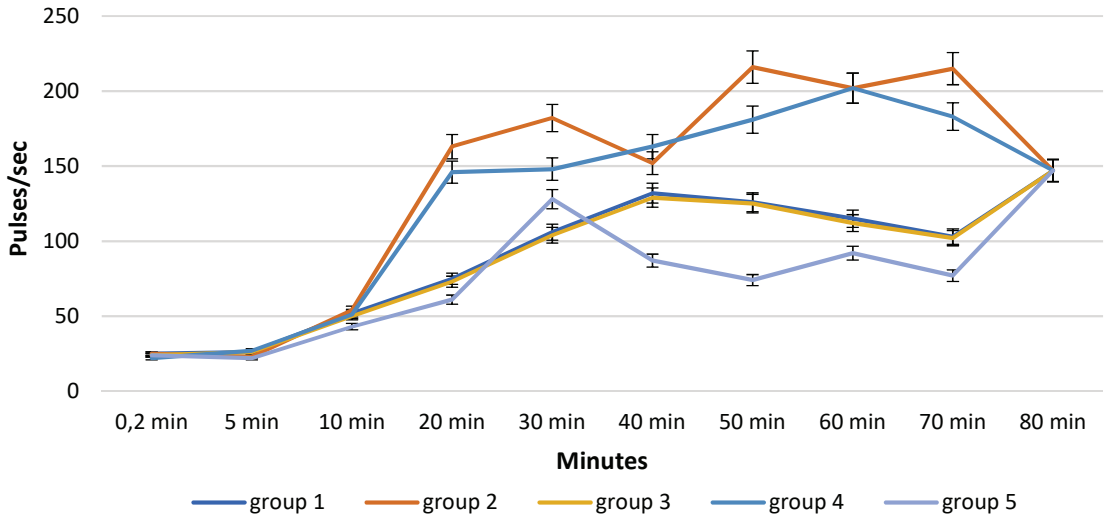

Figure 1. Induced neutrophils chemiluminescence in laboratory animals (n = 3), measurement inaccuracy per each group is no more than 5%

81 pulses/sec or 79.4% ($P \leq 0.001$) at the 60th and 70th minutes after induction, respectively.

The Figure 2 below shows the data of induced chemiluminescence of mice neutrophils on the 7th day of the experiment.

From the data presented, it follows that the level of neutrophil CL obtained from mice treated with bursal peptides (group 2) is higher than the neutrophil CL level of intact animals (group 1). So the number of neutrophil pulses at the 60th, 70th and 80th minutes of induced chemiluminescence against the background of the bursal peptides introduction is higher in comparison with the 1st group by 71 pulses/sec or 63.4% ($P \leq 0.001$), by 107 pulses/sec or 146.6% ($P \leq 0.001$) and by 81 pulses/sec or 103.8% ($P \leq 0.001$), respectively.

The use of bursal peptides among the immunosuppressed mice (group 4) increases the number of neutrophil pulses at 60, 70 and 80 minutes of induced chemiluminescence in comparison with immunosuppressed mice treated per os with saline as a placebo (group 3) by 44 pulses/sec or by 40.0% ($P \leq 0.05$), by 91 imp/sec or by 116.1% ($P \leq 0.001$) and by 39 imp/sec or 51.3% ($P \leq 0.05$), respectively.

The Figure 3 shows the data of induced chemiluminescence of mice neutrophils on the 14th day.

In all groups of animals treated with peptides, the level of neutrophil CL exceeded that of intact animals (group 1). Thus, the induced neutrophils chemiluminescence in the second group was at the 60th, 70th and 80th minutes higher by 8 pulses/sec or 21.1%, by 21 pulses/sec or 47.7% and by 17 pulses/sec or 42.5% ($P \leq 0.05$) in comparison with the first group. In immunosuppressed animals treated with bursal peptides (group 4), neutrophil chemiluminescence induced at the 60th, 70th and 80th minutes was higher by

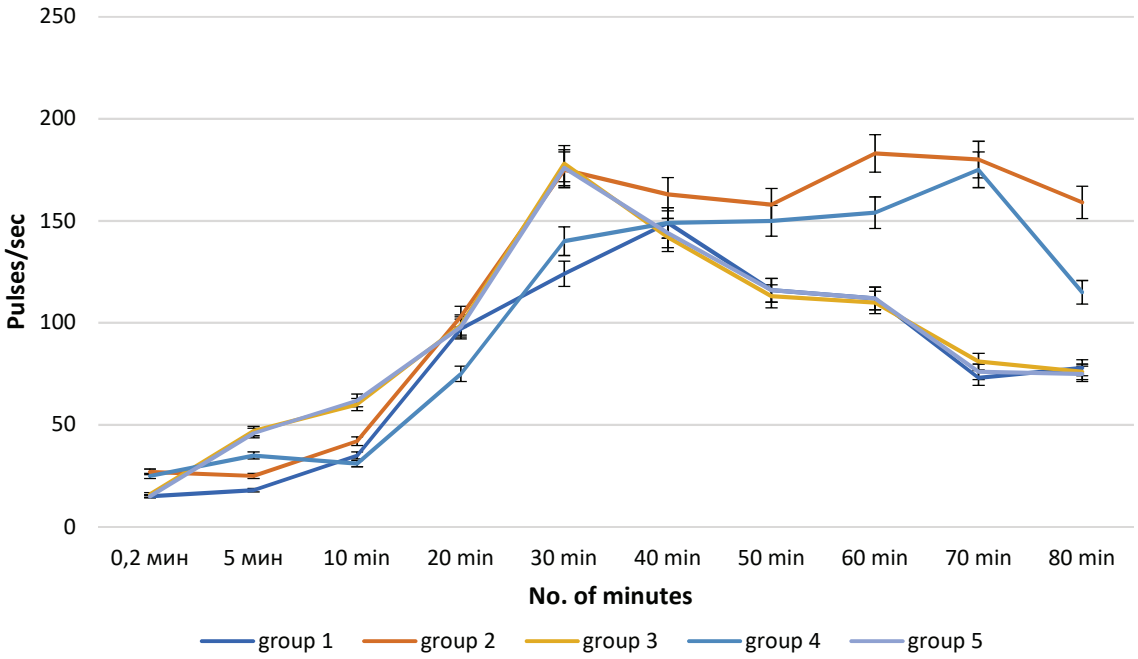

Figure 2. Induced chemiluminescence of neutrophils of laboratory mice (n = 3), measurement error for each group is not more than 5%

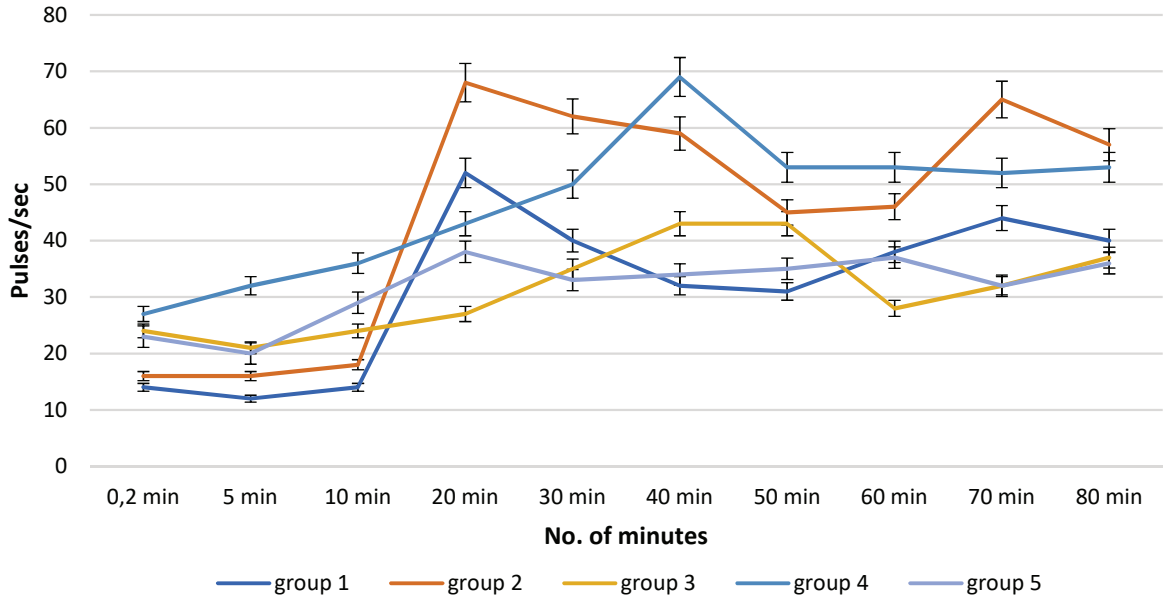

Figure 3. Induced neutrophils chemiluminescence in laboratory mice on the 14th day of the experiment (n = 3), measurement inaccuracy for each group is not more than 5%

25 pulses/sec or 89.3% ($P \leq 0.01$), by 20 pulses/sec or 62.5 ($P \leq 0.05$) and by 16 pulses/sec or 43.2% ($P \leq 0.05$) in comparison with the group of mice treated with saline *per os* as a placebo (the 3rd group). The increase in the amount of induced neutrophils CL in mice treated with cyclophosphan is probably caused by the fact that after a short suppression of immune responses the compensatory immune stimulation response is observed in the body.

The results of the studies indicate that a day before the experiment, a single administration of cyclophosphan to mice leads to a persistent immune disorder. The characteristic signs of immunosuppression were a significant decrease in the number of subpopulations of CD3+, CD22+, CD3+CD8+ lymphocytes in the blood in animals of the third, fourth and fifth groups against the background of the use of cytostatics. The data obtained are consistent with the studies of the authors [13], which showed that immunosuppression in mice can be induced by a single intraperitoneal injection of cyclophosphan at a dose of 100 mg/kg, and in another study of the authors [13], immunosuppression was induced using an intraperitoneal injection of 50 mg/kg of cyclophosphan twice with a break of three days or cyclophosphan at a dose of 125 μg/mouse intraperitoneally one time.

Similar decrease in number of lymphocytes subpopulations in the blood in immunodeficient mice were obtained in the other studies [14].

According to researchers [15,16], biologically active peptides with a medicinal effect, including an immunomodulatory effect, can be obtained by enzymatic and subsequent technological processing of raw materials of animal origin, which is consistent with the technology for obtaining peptides, studied in our experiment.

Peptide-based immunomodulators isolated from the tissues of the immunocompetent organs of animals and poultry, in particular, glucosaminylmuramyl dipeptide (GMDP), registered under the name Likopid® [6], deserve special attention. It was found that the introduction of bursal peptides into immunosuppressed mice allows maintaining the homeostasis of subpopulations of T- lymphocytes and B-lymphocytes, and on the 7th day there is a trend to increasing the number of studied immune cells. The obtained data are consistent with the statement of the authors [17], who ran an experiment on immunization of chickens with bursal pentapeptide-II (BPP-II) and a vaccine against avian influenza virus (AIV) with the determination of antibodies and interleukin-4 production. The results showed that BPP-II plays a strong inducing role in humoral immune responses. To examine gene expression at the transcriptional level, avian B-lymphocyte DT40 cells were treated with BPP-II and analyzed by gene microarray. The obtained results proved that the administration of BPP-II regulates 11 ways in which the homologous recombination is a vital mechanism involved in conversion and diversification of immunoglobulin antibody genes during development of B-cells. These results suggested that the biologically active BPP-II peptide derived from the bursa may be involved in antibody production and development of B -cells.

The obtained data on the activation of T- lymphocytes and B-lymphocytes are consistent with the results of studies [18] where it was found that the bursal peptide BSP-II induces strong production of AIV-specific HI antibodies in immunized chickens and increases the viability of avian pre-B-lymphocytes DT40 cells.

Studies have found that the introduction of peptides into laboratory animals leads to a weakening of the effect of cytostatics and contributes to the activation of the immune system. The data obtained are consistent with the results of studies by the authors [9], who state that bursal peptides have immunosuppressed and antioxidant activity, in particular, the BP5 peptide isolated from the bursa of Fabricius significantly stimulates the expression of the p53 protein in HCT116 colon cancer cells. BP5 has a strong inhibitory effect on cell growth and induces apoptosis in HCT116 cells. Mechanically, BP5 stops the cell cycle in the G1 phase by increasing the expression of p53 and p21 and decreasing the expression of cyclin E1-CDK2 complex. Introduction of BP5 dramatically activates the stress-mediated endoplasmic reticulum (ER) apoptotic pathway, as evidenced by a significant increase of expression of unfolded protein response sensors (IRE1a, ATF6, PERK) as well as downstream signaling molecules (XBP-1s, eIF2a, ATF4, and CHOP) and a significant change in the phenotypic changes induced by BP5 in IRE1, ATF6 and PERK knockout cells.

Our study on assessment of immunomodulating effect of bursal peptides is consistent with the results of studies [19] where the effect of bursopentin (BP5) on the protection of dendritic cells from oxidative stress during immunosuppression was studied. BP5 has shown potent protective effects against lipopolysaccharide (LPS)-induced oxidative stress in dendritic cells, including nitric oxide, reactive oxygen species, and lipid peroxidation. In addition, BP5 increased cellular reduction status by increasing reduced glutathione (GSH) and the GSH/GSSG ratio. Along with this, the activity of some antioxidant redox enzymes, including glutathione peroxidase, catalase and superoxide dismutase, was clearly increased. BP5 also suppressed submucosal maturation of dendritic cells in the LPS-stimulated intestinal epithelial cell co-culture system. As a result, it was found that under the influence of BP5 the concentration of heme oxygenase 1 in LPS-induced dendritic cells significantly increases and plays an important role in suppressing oxidative stress and maturation of dendritic cells. These results indicated that BP5 could protect dendritic cells from LPS-induced oxidative stress and would have potential applications in management of associated inflammatory responses.

We have found that bursal peptides enhance the immune response in the form of a change in the number of subpopulations of B-cells and / or T-cells. In a healthy

body, CD4+ helper T-cells enhance the immune response regulated by CD8+ T-cells, while activated CD8+ T-cells release inflammatory cytokines, leading to death of infected cells. In immunosuppressed patients, CD8+ T-cells are not properly activated by mitogenic stimuli [20]. Therefore, activation of CD8+ lymphocytes by bursal peptides can relieve immunosuppression.

Against the background of immunomodulators application, the immunological studies pay special attention to the study of the chemiluminescence kinetics of neutrophils, as they are the most common leukocytes in the blood circulation and the first cells recruited to infection lesions or inflammation foci. Neutrophils in the blood circulation system are considered to be short-life cells that undergo constitutive apoptosis after 24 hours only. The migration of neutrophils from the blood circulation system into tissues is a multistage process that includes their passage along the vascular endothelium, adhesion to endothelial cells, extravasation through the vascular endothelium, and migration to inflammatory foci [21]. Neutrophilic clearance of microbes occurs by several processes, including phagocytosis, degranulation reactions, generation of reactive oxygen species (ROS), and formation of extracellular traps (networks) of neutrophils [22].

The granules are essential for neutrophils to implement their role in innate immunity. Upon activation of the neutrophils, the granules can release their contents into the immediate microenvironment. There are three types of granules in neutrophils: 1. Azurophilic granules, which are reservoirs of antimicrobial compounds, including myeloperoxidase (MPO), defensins, lysozyme, bactericidal-penetrating protein, neutrophil elastase (NE), and cathepsin. 2. Secondary granules, which are characterized by the glycoprotein lactoferrin, including NGAL and hCAP-18 [23]. 3. Gelatinase granules, which are thought to be the storages for metalloproteases such as gelatinase and leucolysin [23]. In response to infections, neutrophils can lead to pathogens destruction by releasing reactive oxygen species ((ROS) and MPO and NADPH oxidase activity) and reactive nitrogen species ((RNS) and nitric oxide synthase (NOS)) [23]. Despite their beneficial role against pathogens, chronic or uncontrolled production of ROS can contribute to damage of lipid membrane, DNA damage, and genetic instability. Extracellular neutrophil traps (networks), which are generated by activated neutrophils, play a crucial role in the immune system [23]. The networks are made up of cell-free DNA, histones, antimicrobial proteins, dangerous molecules and autoimmune antigens and play a vital role in fighting against bacterial, viral, fungal and parasitic infections. Since the increase in neutrophil activity is an important direction of therapy in immunosuppression according to studies [24], we determined the number of neutrophil pulses during the induced chemiluminescence. The obtained results of neutrophil chemiluminescence reflect the status of the immune defense of laboratory animals. Thus, our functional analysis of the measurement of neutrophil luminescence allows us to confirm that the administration of bursal peptides to immunosuppressed mice contributes to weakening of the cytostatic effect, which is represented by a greater number of neutrophil pulses at the 50^{th} and 70^{th} minutes of induced chemiluminescence in comparison with immunosuppressed mice that got saline *per os* as a placebo. The data obtained are consistent with the results of studies [25] where an increase in neutrophils activity under the action of animal origin peptides was established.

Conclusion

It follows from the analysis of scientific knowledge and the results of our own research in the field of biologically active peptides, obtained from raw materials of animal origin, that the mechanism of peptides action in various pathologies is currently being intensively studied by domestic and foreign scientists.

On the basis of the performed researches it can be assumed that the immunocompetent organs of broiler chickens (bursa of Fabricius) are a promising source of immunotropic peptides. As a result of studies on immunosuppressed mice it was found that intake of peptides isolated from bursa of Fabricius of the broiler chicken into animals increases the functional activity of lymphocyte subpopulations (CD3+, CD22+, CD3+CD4+, CD3+CD8+), which increase evidences an immunotropic effect.

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