RESEARCH ARTICLE

Immunomodulatory Properties of the Cryopreserved Pigskin Substrate in an Experiment

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Abstract

Introduction: Allergic pathology remains a global problem of humanity. Despite a wide range of anti-allergic drugs, the problem of developing reasonable and safe measures for allergic diseases (ADs) treatment and prevention remains unresolved. Among existing modern therapeutic measures, particular attention is drawn to the allergen-specific immunotherapy. It seems promising to use the cryopreserved pigskin substrate (CPSS), cellular elements of which serve as the source of antigens and are capable of triggering immunotolerance mechanisms. We investigated the effect of CPSS on the development of mouse immunodeficiency and an allergic reaction of delayed-type hypersensitivity (DTH). **Materials and Methods:** The effect of CPSS on the development of mouse immunodeficiency induced by hydrocortisone acetate (once, intraperitoneally, 250 mg/kg,) was investigated. The CPSS was administered on a daily basis into the stomach of animals as a suspension emulsified with Tween at dosas 200 mg/kg and 500 mg/kg. As the reference drug, we used an analog regarding pharmacological action, the immunostimulatory agent of natural origin thymalin, lyophilisate for solution for injections (100 mg/kg, intraperitoneally). Blood serum of experimental animals was analyzed to assess parameters of the humoral component of the immune system, namely, titers of hemolysins and hemagglutinins and the number of antibody-forming cells according to Ierne and Nordin. The effect of CPSS on development an allergic reaction of DTH induced by skin sensitization with a 5% 2,4-dinitrochlorobenzene (DNCB) solution (five applications of three drops of a 5% alcohol-acetone solution (1:1) of 2,4-DNCB to the animal skin. Four days after the last application, three drops of the 5% 2,4-DNCB solution were applied to the right paw of an animal (initiation of the allergic reaction) was investigated. As the reference drug, we used loratadine tablets, at a dose of 1 mg/kg. Popliteal lymph nodes were removed and weighed. DTH reaction index was determined. The concentration of IgE, as well as the level of tumor necrosis factor-α (TFN-α), interleukin-2 (IL-2), and IL-10 cytokines, were assessed in blood serum of sensitized animals. Neutrophil phagocytic activity was determined in whole-blood after coincubation of neutrophils with yeast cells. **Results:** The study established that preventive administration of the CPSS contributes to restoring the balance between cellular and humoral immune responses, which results in inhibition of the pathological process, reduction in specific hyperresponsiveness of tissues to proinflammatory mediators, and reduction of signs of allergic inflammation or immunodeficiency states. **Conclusions:** Preventive administration of the CPSS contributes to restoring the balance between cellular and humoral immune responses, which results in inhibition of the pathological process, reduction in specific hyperresponsiveness of tissues to pro-inflammatory mediators, and reduction of signs of allergic inflammation or immunodeficiency states.

Key words: Allergen-specific immunotherapy, allergic diseases, cryopreserved pigskin substrate

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INTRODUCTION

Allergic pathology in both adults and children remains a global problem of humanity. About 25–30% of the pediatric population suffers from allergic diseases (ADs). The relevance of issues of allergy treatment and prevention in children is driven by significant "rejuvenation" (the shift in the disease-onset toward a much younger age) and a tendency toward increased incidence of severe clinical forms of ADs. Despite a wide range of anti-allergic pharmaceutical products, the problem of developing reasonable and safe measures for ADs treatment and prevention remains unresolved.[1]

Among existing modern therapeutic measures, particular attention is drawn to the allergen-specific immunotherapy (ASIT). The ASIT method is based on the ability of the human immune system to switch from an intensive and abnormal response to an allergen toward a tolerant and normal reaction through modification of the antigen-antibody reaction and functional activity of T-lymphocytes. In the ASIT mechanism, the essential role is played by the production of so-called blocking antibodies (Ab): IgG_4Ab and IgA Ab.[2,3] Due to a reduction in the production of interleukin-4 (IL-4) and rise in the interferon-γ (INF-γ) production, the Th_2 phenotype of the immune response changes to the Type 1 helper lymphocytes (Th_1) phenotype.[4] When treatment with high (subshock) doses of the allergen is continued, a condition of allergen-specific anergy is produced in peripheral T-lymphocytes, which is characterized by reduced cell proliferation and release of cytokines, with simultaneous increase in the level of the IL-10 production that inhibits specific T-lymphocytes, decreases the IgE production, and increases the production of IgG_4.[1]

Oral immunotherapy (OIT)[5] is a variety of ASIT. OIT is based on mechanisms of oral immunological tolerance, i.e., a state of active immunological is an activity to an antigen, which the body previously contacted with, when it was administered by the enteral route.[6,7] Induction of oral tolerance occurs after the first contact of the antigen with gut-associated lymphoid tissue (GALT). Antigens interact directly with GALT or affect the immune system after absorption.[8] In the course of treatment with allergens, the number of mast cells and basophils of mucous membranes decreases, the release of pro-inflammatory mediators reduces, and tissue accumulation of eosinophils and neutrophils is inhibited. ASIT is considered to be the only pathogenetically substantiated method for ADs treatment.[9,10] Experimental and clinical studies show sufficient efficacy of the method of oral immunological tolerance in autoimmune diseases, such as rheumatoid arthritis, disseminated sclerosis, atopic dermatitis, and diabetes mellitus.[11]

Regarding ASIT, the use of the cryopreserved pigskin substrate (CPSS) seems promising. In this case, cellular elements of the pigskin (elastin, reticular, and collagen fibers of the dermal connective tissue) serve as a source of antigens and are capable of triggering immunotolerance mechanisms.

The purpose of this study was to investigate immunomodulatory properties of the CPSS in a mouse model of immunodeficiency induced by hydrocortisone acetate (HA), and in the setting of delayed-type hypersensitivity (DTH) in rats induced by 2,4-dinitrochlorobenzene (DNCB).

MATERIALS AND METHODS

Animal studies were conducted in compliance with the basic provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 18. 3. 1986), Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations, and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes, Order of the Ministry of Health of Ukraine from February 13, 2006 No. 66, Law of Ukraine "On protection of animals from cruelty" (2006).[12] During the experiment, animals were kept in a vivarium at 18–24°C and humidity of 50–60%, with a natural "day-night" light regime, in plastic cages, receiving balanced diet according to the current regulations.

Investigation of immunomodulatory properties of the CPSS was carried out in a mouse model of immunodeficiency induced by intraperitoneal single administration of HA (Hydrocortison-Richter, Budapest, Hungary) at a dose of 250 mg/kg.[13]

The following animal groups, consisting of 8 animals each, were used: Group 1 was intact control and included healthy animals administered with drinking water; Group 2 represented control pathology and included animals administered with HA at a dose of 250 mg/kg; Groups 3 and 4 animals were administered with the CPSS at doses of 200 and 500 mg/kg in the setting of HA administration; Group 5 animals received a reference drug, thymalin, at a dose of 100 mg/kg in the setting of HA administration.

The CPSS was administered on a daily basis into the stomach of animals as a suspension emulsified with Tween for 5 days until production of immunodeficiency and intragastrically once daily throughout the experiment. As the reference drug, we used an analog regarding pharmacological action, the immunostimulatory agent of natural origin thymalin, lyophilisate for solution for injections produced by BIOFARMAPrAT, Kyiv, Ukraine. The reference drug was intraperitoneally administered to animals at a dose of 100 mg/kg according to the similar regimen as the study drug. The total period of administration of each of the drugs was 11 days. The dose of thymalin was calculated according to Freireich *et al*. based on the average daily dose for humans about the body surface area and coefficients of species sensitivity for mice.[14]

To assess the degree of immunodeficiency and efficacy of the studied products, 1 day after administration of HA, mice were

intraperitoneally immunized with a 3% suspension of sheep red blood cells at a dose of 0.2 mL/20 g body weight of mice.

On day 11, animals were removed from the experiment by decapitation under inhalation anesthesia (chloroform). Blood serum of experimental animals was analyzed to assess parameters of the humoral component of the immune system, namely, titers of hemolysins (HLs) and hemagglutinins (HAs) according to the method of serial dilutions in polystyrene,[15] and the number of antibody-forming cells (AFCs) according to Ierne and Nordin.[16]

In addition, spleen cellularity was estimated. The spleen was weighed using a torsion balance, and its relative weight (weight coefficient [WC]) was calculated. After the disintegration of the lymphoid organ using a tissue grinder, the number of splenocytes was counted, and their concentration in suspension was determined in the Goryaev chamber according to the conventional method.[15,17] The body weight gain was used as an integral indicator of the general physiological condition of mice.

The effect of the CPSS on the development of the allergic reaction of DTH was studied in the second set of experiments in the setting of skin sensitization with DNCB. Experiments were carried out in outbred male rats. The following groups, consisting of 7 animals each, were used: Group 1 was intact control (animals that received no drugs); Group 2 was control pathology (animals without pharmacological correction that were sensitized with skin applications of a 5% DNCB solution; Group 3 included experimental animals that received the intragastric CPSS at a dose of 200 mg/kg in the setting of sensitization with 2,4-DNCB; and Group 4 included experimental animals that were intragastrically administered with a reference drug, loratadine tablets, at a dose of 1 mg/kg in the setting of sensitization with 2,4-DNCB.

The study drugs were administered according to the preventive regimen for two weeks and during the whole sensitization period by intragastric route as a suspension with Tween. Rats were sensitized by five applications of 3 drops of a 5% alcohol-acetone solution (1:1) of 2,4-DNCB to the animal skin. Four days after the last application, three drops of the 5% 2,4-DNCB solution were applied to the right paw of an animal (initiation of the allergic reaction). 3 drops of alcohol-acetone solution (1:1) were applied to the contralateral paw. After 24 h (at the maximum level of DTH reaction development), animals were sacrificed by decapitation under ether anesthesia. Blood was collected for serum preparation. Popliteal lymph nodes (PLNs) were removed and weighed. By the difference in PLN weight, the DTH reaction index was determined, which characterizes the severity of allergic inflammation:

$$RI = \frac{W_{exper} - W_{contr}}{W_{contr}} \times 100$$

Where W_{exper} is the PLN weight of the experimental paw; W_{contr} is the PLN weight of the control paw.

Neutrophil phagocytic activity was determined in whole blood after coincubation of neutrophils with yeast cells for 45 min 37°. The following parameters were determined: Phagocytic index (PI), a percentage of phagocytizing cells per 100 cells was calculated; phagocytic number (PN), an average number of yeast cells absorbed by one neutrophil was calculated16).

The concentration of IgE, as well as the level of tumor necrosis factor-α (TFN-α), IL2, and IL10 cytokines, were assessed in blood serum of sensitized animals. The concentration of IgEwas determined in blood serum using a test system produced by NVL Granum (Ukraine) according to the sandwich method (two-site enzyme-immunoassay). The serum level of TFN-α, IL2, andIL10 cytokines was determined using an enzyme immunoassay kit produced by ZAO Vector-Best, Novosibirsk (Russia).

The obtained experimental data were processed according to methods of various statistics using the Statistica 6.0 statistical software package. The obtained data were expressed as the arithmetic mean and its standard error *(M±m)*, the median and the upper and lower quartile *(Me (Q25; Q75),* or the arithmetic mean and sample minimum and sample maximum *(M (min; max)*. The normality of distribution of quantitative parameters was checked using the Shapiro–Wilk test. Regarding data not subject to the law of normal distribution, the Mann–Whitney test was applied for intergroup comparison; and the Newman–Keuls method was used for data with normal distribution. The critical value of the significance level was taken as $P < 0.05$.

RESULTS OF THE RESEARCH AND THEIR DISCUSSION

The effect of the CPSS on the development of mouse immunodeficiency induced by HA

The obtained data showed that administration of HA in mice from the control pathology group caused reduction by a factor of 3 in the number of AFCs, reduction by a factor of 3 in the HA titer, and by a factor of 2 in the HL titer [Tables 1 and 2]. In parallel to this, reduction by 34% in the body weight and reduction by a factor of 2 in the spleen weight of mice were observed, whereas the number of splenocytes was reduced by a factor of 5 [Tables 3 and 4]. However, a variation of these parameters in the group of intact animals remained within the physiological normal ranges [Tables 1 and 2]. These findings reveal the immunosuppressive action of HA in mice.

Preventive administration of the CPSS reduced the toxic action of HA: Body weight reduction was lower than in the

Table 1: Effect of the CPSS on the number of AFCs in mouse spleen in the setting of immunodeficiency induced by HA, *Me*(Q25; Q75)

Animal group	n	The number of AFCs per spleen
Intact control	8	1840 (1680; 1920)
Control pathology	6	880 (640; 960)*
CPSS, 200 mg/kg	8	1280 (1040; 1840)**
CPSS, 500 mg/kg	8	1280 (1160; 1400)*/**
Thymalin, 100 mg/kg	8	1680 (1280; 2000)**

*Differences are statistically significant regarding values of negative control, $P<0.05$; **differences are statistically significant regarding values of control pathology, $P<0.05$. CPSS: Cryopreserved pigskin substrate, AFCs: Antibody-forming cells, HA: Hydrocortisone acetate

Table 2: Effect of the CPSS on titers of HLs and HAs in mouse blood serum in the setting of immunodeficiency induced by HA, *Me*(Q25; Q75)

Animal group	n	Indicators	
		HAs, $\log_2$	HLs, $\log_2$
Negative control	8	12 (12;12,5)	10,5 (9; 13)
Control pathology	8	4 (3; 6)*	5 (4; 7)*
CPSS, 200 mg/kg	8	12 (11; 13)**	12,5 (12; 13)*/**#
CPSS, 500 mg/kg	8	12 (11,5; 12)**	10 (7; 10)*/**/#
Thymalin, 100 mg/kg	8	11 (11; 12)**	15 (14; 15)*/**

*Differences are statistically significant regarding values of negative control, $P<0.05$; **differences are statistically significant regarding values of control pathology, $P<0.05$; #differences are statistically significant regarding values of the thymalin reference drug, $P<0.05$. CPSS: Cryopreserved pigskin substrate, HAs: Hemagglutinins, HA: Hydrocortisone acetate, HLs: Hemolysins

control pathology group and had no statistical difference from values obtained for intact control [Table 1]. However, the relative spleen weight and the number of splenocytes remained at the level of control pathology [Table 2].

The reference drug, thymalin, exerted an action that was similar regarding severity and the tendency of changes to the study drug, the CPSS, at a dose of 200 mg/kg and 500 mg/kg. No statistically significant differences were observed between the values of parameters of study drugs [Tables 1-4].

The effect of the CPSS on the development of the allergic reaction of DTH induced by skin sensitization with a 5% DNCB solution in rats

It was established that the allergic reaction causes predictable changes of the immunological status of animals. Phagocytosis intensity (PI, showed a clear tendency toward reduction, while the absorbing capacity of neutrophils [PN – FU, Table 5] was significantly suppressed.

Determination of cytokine profile showed a statistically significant increase in TNF-α by 26% and IL-2 by 9% in blood serum of sensitized animals, which suggests activation of the cell-mediated response [Table 6]. At the same time, statistically significant increase in the concentration of IL-10 by 15% and the sensitization severity marker IgE was observed [Table 6].

As the result of these changes, the DTH reaction index increased by a factor of 8 in the group of sensitized animals (positive control) compared to parameters observed in animals from the intact control group [Table 7].

Under the action of the CPSS, neutrophil phagocytic activity was reported to have restored to the level of intact animals [Table 5]. Measurement of the serum concentration of cytokines produced by Type 1 and Type 2 T-helpers showed that the CPSS effect caused a reduction in the level of TFN-α, IL-2, and IgE, while the IL-10 level increased even more than

Table 3: Effect of the CPSS on body weight changes over time in mice with immunodeficiency induced by HA, *Me*(Q25; Q75)

Animal group	n	Differences between baseline values of body weight and values at the end of the experiment	
		g	%
Intact control	8	–2.5 (–3.0; –1.5)	–9 (–13; –6)
Control pathology (HA)	6	–9.5 (–11.0; –7.0)*	–34 (–36; –32)*
CPSS, 500 mg/kg+HA	8	–4.5 (–6.5; –3.0)**	–21 (–22; –11)**
CPSS, 500 mg/kg + HA	8	–2.0 (–3.0; –0.5)**	–8 (–13; –2)**
Thymalin, 0.1 ml/kg	8	–2.5 (–4.0; –1.0)**	–11 (–17; –4)**

*Differences are statistically significant regarding values of intact control, $P<0.05$; **differences are statistically significant regarding values of control pathology, $P<0.05$; n: Number of animals in each group. CPSS: Cryopreserved pigskin substrate, HA: Hydrocortisone acetate

Table 4: Effect of the CPSS on the WC and cellularity of the spleen in mice with immunodeficiency induced by HA, *Me(Q25; Q75)*

Animal group	n	Spleen WC, %	Number of splenocytes, 10^9/L
Intact control	8	0.60 (0.44; 0.85)	555 (400; 680)
Control pathology (HA)	6	0.34 (0.22; 0.35)*	110 (90; 130)*
CPSS, 200 mg/kg+HA	8	0.29 (0.15; 0.41)*	155 (90; 270)*
CPSS, 500 mg/kg+HA	8	0.15 (0.11; 0.24)*	80 (40; 115)*
Thymalin, 0.1 ml/kg	8	0.23 (0.22; 0.24)*	100 (90; 160)*

*Differences are statistically significant regarding values of intact control, $P<0.05$; n: Number of animals in each group.
CPSS: Cryopreserved pigskin substrate, HA: Hydrocortisone acetate, WC: Weight coefficient

Table 5: Effect of the CPSS on neutrophil phagocytic activity in the setting of the DTH reaction induced by the 5% DNCB solution in rats (*n*=7), *M (min; max)*

Animal group	FI	FU
Intact control	21,5 (18; 28)	18,5 (13; 30,9)
Positive control (DNCB)	16,1 (5; 23)	10,8 (3,2; 15,9)*
DNCB+CPSS, 200 mg/kg	23,6 (18; 31)	24,0 (18,3; 31,3)**
DNCB+Loratadine, 1 mg/kg	21,7 (8; 40)	25,8 (9,9; 56,3)**

*Differences are statistically significant regarding values of intact control, $P<0.05$; **differences are statistically significant regarding values of positive control, $P<0.05$. CPSS: Cryopreserved pigskin substrate, DNCB: 2,4-dinitrochlorobenzene, DTH: Delayed-type hypersensitivity

in the positive control group [Table 6]. The DTH reaction index was restored to the level of normal nonsensitized animals. It should be noted that changes in these parameters caused by CPSS were statistically significant in comparison with the same parameters in the group of sensitized animals [Table 7].

Under the action of the reference drug, loratadine, there was a statistically significant reduction in concentrations of TFN-α and IL-2 cytokines produced by Th_1, and normalization of levels of IgE and IL-10 released with Th_2 [Table 6]. The delayed-type reaction index reduced by a factor of 3 compared to the value of positive control but remained higher than in the intact animal group. These changes were statistically significant [Table 7].

Affirmative data from previous studies, suggesting an immunostimulatory action of the CPSS in animals with normal immune status,[18] justified determination of the drug efficacy in the setting of experimental pathology associated with immune system imbalance. In experimental settings, the modeled imbalance can be induced by several drugs, including HA used in this case. It is an established and universally accepted fact that hormonal loading with glucocorticoids causes immunosuppressive effect, which provided grounds for their use as model drugs.[19] Unlike cytostatics, their immunosuppressive effect occurs using suppression of the immune response at different stages of its formation. They dramatically inhibit migration of stem cells, T- and B- lymphocytes from the bone marrow and the thymus to blood, and suppress cooperation of immunocompetent cells. Current evidence suggests that hydrocortisone suppresses the release of cytokines (ILs-1, 2 and INF) from lymphocytes and macrophages as well as the release of inflammation mediators from eosinophils, and reduces the metabolism of arachidonic acid toward the formation of pro-inflammatory prostaglandins.[20-22]

This study has shown that administration of HA in mice from the control pathology group caused development of immunodeficiency, which was reflected by decreased antibody synthesis [Tables 3 and 4], and was accompanied by reduction in both the body weight and WC of the mouse spleen [Tables 1 and 2], which is an expected result of cytotoxic and immunosuppressive actions of high doses of hydrocortisone.[13]

Preventive administration of study drugs reduced the toxic action of HA: Decrease in the body weight was significantly less marked than in the control pathology group and had no statistically significant differences from values of intact control [Table 1]. However, the relative spleen weight and the level of cellularity remained at the level of control pathology [Table 2]. The reference drug, thymalin, exerted an action that was similar in terms of severity and the tendency of changes to the study drug, the CPSS, at a dose of 200 mg/kg and 500 mg/kg; no differences were observed between values of parameters of study drugs [Tables 1 and 2].

It is generally acknowledged that immunity classification into the cellular and the humoral component is rather conditional. Since the process of antibody synthesis by B-lymphocytes is impossible without the participation of main regulators of the immune system, T-helpers, and T-suppressors, the immune response is the result of cooperative interaction between T- and B-lymphocytes. Thus, reduction in the number of AFCs induced by HA reflects the functional state and capabilities of both humoral and cellular components of immunity.[23]

Preventive intragastric administration of the CPSS contributed to restoring the immunoresponsiveness of animals: HL and HA titers were at the level of intact animals, and the number of AFCs was significantly higher than in the control pathology group but lower than in intact animals [Tables 3 and 4]. Efficacy of the CPSS was the same at both doses studied, 200 mg/kg and 500 mg/kg, and did not differ

Table 6: Effect of the CPSS on concentrations of cytokines and IgE in blood serum of rats in the setting of the DTH reaction induced by the 5% DNCB solution, $M \pm m$ (n=7)

Animal group	The content of cytokines, pg/ml			The content of IgE, pg/ml
	TFN-α	IL-2	IL-10	
Intact control	36.84±0.11	81.96±0.72	59.59±1.45	110.76±2.21
Positive control (DNCB)	46.35±1.16*	89.38±0.83*	68.79±0.98*	134.03±5.53*
DNCB+CPSS, 200 mg/kg	37.80±0.36**	81.15±0.89**	75.82±2.51*/**/#	115.92±2.97**
DNCB+Loratadine, 1 mg/kg	38.58±0.52*/**	82.15±0.86**	66.28±1.09*	111.41±3.02**

*Differences are statistically significant regarding values of intact control, P<0.05; **differences are statistically significant regarding values of positive control, P<0.05; #differences are statistically significant regarding values of drug comparison tablet "Loratadine," P<0.05. CPSS: Cryopreserved pigskin substrate, DNCB: 2,4-dinitrochlorobenzene, DTH: Delayed-type hypersensitivity, TFN-α: Tumor necrosis factor-α, IL: Interleukin

Table 7: Effect of the CPSS on the development of the DTH reaction induced by the 5% DNCB solution in rats, M $(min; max)$, n=7)

Animal group	Reaction index
Intact control	5.6 (2.0; 11.4)
Positive control (DNCB)	45.1 (27.6; 77.8)*
DNCB+CPSS, 200 mg/kg	11.8 (2.6; 28.6)**
DNCB+Loratadine, 1 mg/kg	14.4 (5.3; 23.4)*/**

*Differences are statistically significant regarding values of intact control, P<0.05; **differences are statistically significant regarding values of positive control, P<0.05. CPSS: Cryopreserved pigskin substrate, DNCB: 2,4-dinitrochlorobenzene, DTH: Delayed-type hypersensitivity

significantly from the reference drug, thymalin, with the exception of the HL parameter that was higher than in the negative control group and all experimental groups.

It should be noted that unlike the reference drug, thymalin, which reveals excessive immunostimulatory action, the use of the CPSS caused the only recovery of parameters to the level of intact animals. In other words, the obtained data suggest that the CPSS action can be considered more physiological, involving no excessive stimulation of the immune system, which may subsequently allow avoiding development of autoimmune responses.

The purpose of the next stage of work was to investigate the effect of the CPSS on the development of the allergic reaction of DTH induced by skin sensitization with the 5% DNCB solution in rats. The final application of the 2,4-DNCB allergen to paws of pre-sensitized animals triggered the effector phase of the immune response with subsequent initiation of cellular reactions aimed at neutralizing and destroying the antigen. Determination of the cytokine profile showed statistically significant increase in the concentration of TNF-α and IL-2, which suggests activation of a population of Th_1 that mediate the cellular immunity. At the same time, significant increase in the level of IL-10 was reported. Since the main function of this cytokine is to increase expression of Class II MHC (major histocompatibility complex) and suppress production of pro-inflammatory cytokines,[23] rise in blood levels of IL-10 in positive control animals in this model pathology can be considered as compensatory response to Th_1 activation. In addition, not well-marked, but statistically significant increase in the level of the Ig E sensitization severity marker was observed [Table 6], which was associated with activation of allergen-specific cells that produce specific Ab in response to the allergen. These changes caused an increase by a factor of 8 in the DTH reaction index in the group of sensitized animals compared to parameters of animals from the intact control group [Table 7]. At the same time, significant changes in the immunological status of animals were reported. The intensity of phagocytosis in the positive control group showed a clear tendency toward reduction: the absorbing capacity of neutrophils was significantly suppressed [Table 5].

Preventive administration of the CPSS resulted in the restoration of neutrophil phagocytic activity to the level of intact animals [Table 5]. Determination of the level of serum cytokines produced by Type 1 and Type 2 T-helpers revealed that under the action of the CPSS, levels of TFN-α and IL-2 decreased, whereas the IL-10 level increased even more than in the positive control group [Table 6]. The last indicates more active anti-inflammatory processes, resulting in a reduction of the concentration of IgE and DTH reaction index down to the level of non-sensitized animals. It should be noted that changes in these parameters caused by CPSS were statistically significant in comparison with the same parameters of sensitized animals from the positive control group.

Under the action of the reference drug, loratadine, positive changes of the studied parameters were also observed, which caused a reduction in intensity of allergic inflammation [Tables 5-7]. Reduction in the concentration of cytokines produced by Th_1 and normalization of levels of IgE and IL-10 released with Th_2 [Table 6] was observed in our study, which corresponds to literature data. It is known that the key mechanism of the anti-allergic action of loratadineis-blocking of histamine H_1 receptors on endothelial cells of postcapillary venules, which results in reduction of vascular permeability, exudation of fluid, proteins, and cytokines determined by pathological impact of histamine that is released from mastocytes and basophils in large quantities

during the early phase of an allergic reaction. These pharmacological properties ensure the high efficacy of the drug in ADs associated with underlying immediate-type hypersensitivity reactions.[24,25] However, it was established that loratadine suppresses chemotaxis of eosinophils[26] and inhibits expression of IFN-γ induced by IL-12, as well as expression of IL-5induced by IL-4.[27] Most likely, it is the effect on both Th_1- and Th_2-dependent mechanisms of allergic inflammation, which explains the efficacy of loratadine in the experimental model of DTH.

Analysis of the obtained results allowed for concluding that the main mechanism of the allergic action of the CPSS in this model is suppression of cellular reactions as evidenced by reduction in the level of Th_1-dependent cytokines and increase in the level of IL-10, a powerful anti-inflammatory cytokine with immunomodulatory properties. IL-10 suppresses the expression of pro-inflammatory cytokines, chemokines, and other inflammatory mediators, thereby performing an important function for inhibition of the allergic reaction.[23] In addition, normalization of neutrophil phagocytic activity and reduction in the level of IgE was observed under the action of the reference drug.

CONCLUSIONS

Preventive administration of the CPSS contributes to restoring the balance between cellular and humoral immune responses, which results in inhibition of the pathological process, reduction in specific hyperresponsiveness of tissues to pro-inflammatory mediators, and reduction of signs of allergic inflammation or immunodeficiency states.

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