EXPERIMENTAL RESEARCHES

PATHOLOGICAL CHANGES OF THE SPLEEN IN MICE INFECTED WITH INFLUENZA AGAINST THE BACKGROUND OF THE USE OF SAPONIN TAUROSIDE Sx1

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ABSTRACT

Background. *It is well known that viral infections are able to cause an imbalance of the interferon system and inhibition of cellular and phagocytic reactions of the body. One of the possible solutions of the flu treatment problem may be the application of immunomodulators of native plant origin since the influenza virus possesses a suppressive effect on cellular immunity and the interferon system.*

The aim. *To evaluate the effect of saponin tauroside Sx1 obtained from Crimean ivy leaves on histological changes in the spleen of mice infected with influenza A/WSN/1/33(H1N1) virus.*

Material and methods. *We used 78 male BALB/c mice weighing 16–18 g which were divided into the groups: control (K; n = 12); healthy animals treated with saponin (KS; n = 22); animals infected with influenza virus A/WSN/1/33(H1N1) (V; n = 22); infected animals treated with saponin tauroside Sx1 twice a day for 3 days (SV; n = 22). Histological studies of the spleen were performed on the days 4 (subgroups V, SV, KS) and 14 (2V, 2SV, 2KS).*

Results. *The spleen tissue of the KS subgroup demonstrated hyperplasia of the white pulp in the form of lymphoid nodules expansion. On the days 4 in the KS subgroup a statistically significant increase in the total area of the lymphoid nodules by 3.9 times compared to the K subgroup was observed. In subgroup V, there was a sharp decrease in the area of white pulp. In subgroup 2V, areas of lymphoid nodules were almost indistinguishable. Applied correction in the SV and 2SV subgroups significantly ceased the damaging effect of the virus: the lymphoid nodules area increased by 2.7 times in the 2SV subgroup compared to 2V.*

Conclusion. *Infection with H1N1 influenza virus leads to a compensatory activation of the immune response, however, on the day 14 a pronounced depletion of the white pulp of the spleen is observed. The introduction of saponin tauroside Sx1 enhanced the functional activity of the spleen due to an increase of the white pulp area.*

Key words: *triterpenoid saponin, influenza virus, spleen, immunomodulation*

Received: 27.04.2022
Accepted: 19.01.2023
Published: 02.03.2023

For citation: Malygina V.Yu., Sataieva T.P., Makalish T.P., Kalfa M.A., Kubyshkin A.V., Rybalko S.Yu., Kirsanova M.A. Pathological changes of the spleen in mice infected with influenza against the background of the use of saponin tauroside Sx1. *Acta biomedica scientifica*. 2023; 8(1): 228-238. doi: 10.29413/ABS.2023-8.1.23

ПАТОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ СЕЛЕЗЁНКИ У МЫШЕЙ, ЗАРАЖЁННЫХ ГРИППОМ, НА ФОНЕ ПРИМЕНЕНИЯ САПОНИНА ТАУРОЗИДА Sx1

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РЕЗЮМЕ

Обоснование. *Известно, что вирусные инфекции вызывают дисбаланс системы интерферонов, угнетение клеточных и фагоцитарных реакций организма. Одним из возможных решений проблемы лечения гриппа может явиться применение отечественных иммуномодуляторов растительного происхождения, поскольку вирусы гриппа оказывают супрессивное действие на клеточный иммунитет и систему интерферонов.*

Цель исследования. *Оценить влияние перорального введения сапонина таурозида Sx1, полученного из листьев крымского плюща, на гистологические изменения селезёнки мышей, заражённых вирусом гриппа A/WSN/1/33(H1N1).*

Материал и методы. *Использовали 78 самцов мышей линии BALB/c весом 16–18 г, разделённых на группы: контрольная, здоровые животные (K; n = 12); контрольная, здоровые животные, получавшие сапонин (KS; n = 22); животные, зараженные вирусом гриппа A/WSN/1/33(H1N1) (V; n = 22); животные, зараженные вирусом гриппа A/WSN/1/33(H1N1) и получавшие сапонин таурозид Sx1 дважды в день в течение 3 дней после заражения (SV; n = 22). Гистологические исследования селезёнки проводили на 4-й (подгруппы V, SV, KS) и 14-й день (2V, 2SV, 2KS).*

Результаты. *В ткани селезёнки подгруппы KS отмечалась выраженная гиперплазия белой пульпы в виде расширения лимфоидных узелков. На 4-й день в подгруппе KS наблюдалось статистически значимое увеличение общей площади лимфоидных узелков по сравнению с подгруппой K в 3,9 раза. В подгруппе V отмечалось резкое уменьшение площади белой пульпы. В подгруппе 2V зоны лимфоидных узелков были практически неразличимы. На фоне коррекции в подгруппах SV и 2SV повреждающее воздействие вируса было выражено значительно меньше: площадь лимфоидных узелков увеличивалась в 2,7 раза в подгруппе 2SV по сравнению с 2V.*

Заключение. *Инфицирование вирусом гриппа H1N1 приводит к компенсаторной активации иммунного ответа, однако на 14-е сутки наблюдается выраженное истощение белой пульпы селезёнки. Введение сапонина таурозида Sx1 положительно влияет на функциональную активность селезёнки за счёт прироста площади белой пульпы.*

Ключевые слова: *тритерпеновый сапонин, вирус гриппа, селезёнка, иммуномодуляция*

Статья получена: 27.04.2022
Статья принята: 19.01.2023
Статья опубликована: 02.03.2023

Для цитирования: Малыгина В.Ю., Сатаева Т.П., Макалиш Т.П., Кальфа М.А., Кубышкин А.В., Рыбалко С.Ю., Кирсанова М.А. Патологические изменения селезёнки у мышей, заражённых гриппом, на фоне применения сапонина таурозида Sx1. *Acta biomedica scientifica*. 2023; 8(1): 228-238. doi: 10.29413/ABS.2023-8.1.23

INTRODUCTION

Influenza, like other acute respiratory viral infections (ARVI), undoubtedly occupies a leading place in the structure of respiratory infectious diseases. According to the World Health Organization (WHO), up to 646,000 patients die from seasonal influenza worldwide every year [1].

During the pandemic of a new coronavirus infection, the danger of influenza should not be underestimated. Influenza remains one of the most important health problems and poses a serious threat to adults and children in all countries [2]. Assessments indicate that annual influenza can affect 5–20 % of adults and 20–30 % of children, and when pandemics occur the incidence of influenza can rise up to 50 % [2]. Influenza complications are also known to be at high risk among young children during the first years of life, adults aged 65 years and over, pregnant women and people with chronic medical conditions, which is due to the immunosuppressive effect of the influenza virus, which aggravates the severity of the existing chronic somatic nosology [2]. Every year the influenza virus mutates, becoming resistant to many antiviral drugs, which also leads to severe complications and lethal outcomes. Chronic cardiovascular or pulmonary disease, obesity, pregnant women and tobacco smokers have been shown to increase the risk of being fatally ill with influenza tenfold [3–5].

Etiotropic anti-influenza drugs are the basis of antiviral chemotherapy, but their number is very limited. According to the clinical guidelines of the Ministry of Health of Russia "Influenza among adults", four drugs are distinguished as antiviral drugs with direct antiviral effect: oseltamivir, zanamivir, riamilovir and umifenovir [6]. Considering the widespread resistance of current strains to adamantane-based drugs and the high likelihood of resistance to neuraminidase inhibitors, as well as the severe side-effects of commonly used antiviral drugs, the search for effective and safe immunostimulants and antivirals to prevent and treat influenza is vital. Such drugs should not be significantly affected by viral variability and should have universal antiviral properties. After the acute and viraemia have subsided among severe influenza patients, the use of immunomodulators, including herbal supplements, to stimulate immune activation and seroconversion is recommended [7, 8].

Saponins make up an extensive group of plant glycosides that inhibit the development of fungi, bacteria, viruses and protozoa, stimulate humoral and cell-mediated immunity, and are also able to enhance the immune response during vaccination [9–11]. When studying the properties of saponins isolated from Crimean ivy *Hedera taurica* (Hibberd) Carrière, it was proved that tauroside H_2, -St-K and -I can enhance antibody synthesis in mice, and tauroside Sx1 is able to inhibit the growth of fungi of the genus *Candida* and enhance the resistance of mice to fungal infection [12, 13]. Oral administration of 200 μg of tauroside Sx1 to mice infected with the influenza virus has been proven to cause a 1.5-fold increase in their survival compared to the survival of infected mice in the control [14]. At the same time, the tissue, cellular and molecular mechanisms of the development of the immune response induced by saponins, as well as their effect on the immunogenesis organs, remain practically unstudied.

The spleen is a highly organised and the largest polyfunctional peripheral organ of the immune system, in which the intensity of immune and filtration processes closely correlates with the architectonics of its white and red pulps as well as the quantitative ratio of the areas occupied functionally by the different segments with their cellular composition. At the same time, the spleen shows a high degree of reactivity with cytological restructuring of its immunocompetent structures under the influence of various endogenous and exogenous factors, including the background of infection. The absence of the spleen is known to cause increased susceptibility to systemic bacterial infections, while the role of the spleen in antiviral immunity is less well studied [15].

Data from other researchers indicate that the rat spleen is able to respond to the introduction of immunomodulators with pronounced morphological changes, which, as a rule, is accompanied by hyperplasia of B-dependent zones [16]. Thus, the choice of the spleen as an experimental model for evaluating the effectiveness of immunomodulatory effects is justified by the fact that it participates in almost all immune and hematopoietic processes, being the center of antigen-dependent proliferation and differentiation of components of both cellular and humoral immune response, its activation, as well as the production and secretion of specific immunoglobulins.

THE AIM OF THE STUDY

To evaluate the effect of oral administration of saponin tauroside Sx1, obtained from Crimean ivy leaves, on histological changes in the spleen of mice infected with influenza A/WSN/1/33(H1N1) virus at different periods of influenza infection.

MATERIALS AND METHODS

Influenza virus (IV) A/WSN/1/33(H1N1) adapted to mice was used in this study. The initial strain of IV was obtained from the collection of the D.I. Ivanovsky Research Institute of Virology of the Russian Academy of Medical Sciences (Moscow). The adaptation of IV to mice and the production of a lethal strain were carried out with repeated passaging of the virus through the lungs of the animals [17]. The initial virus-containing liquid was injected into mice intranasally under light ether anesthesia. After 3 days, the lungs were extracted from the animals and lung tissue homogenate was obtained under sterile conditions, after centrifugation, the supernatant fluid was injected intranasally into the mice. The procedure was repeated three times, the last lung homogenate was injected into 10-day-old chicken embryos in order to accumulate the virus. After six series of similar passages made on mice and chicken embryos, the resulting allantois fluid contained an influenza virus that was lethal to mice. The virus content in the samples was tested

by haemagglutination reaction with chicken erythrocytes [17]. The adaptation of the virus to reproduce in the lungs of mice resulted in a lethal influenza model, which was subsequently used in our studies. In hemagglutinin yield reduction assay of chicken erythrocytes, the dynamics of accumulation of antihemagglutinin antibody titers in the sera of infected animals on the days 4, 7, 14 and 18 after infection was determined. The dynamics of influenza virus accumulation in the lungs of mice on days 2–6 after infection was also studied. An anti-haemagglutinin antibody titer was found to be 80 ± 15.1 on day 4 post-IV infection. The infectious titer of the virus in the lungs of mice at the same time of infection was 2.2×10^3. By the day 14 of the experiment, the antibody titer increased almost 4 times and amounted to 213.3 ± 53.3. The average lifespan of mice during experiments ranged from 9.9 ± 1.3 to 11.5 ± 2.1 days, depending on the weight and age of the animals [14].

In the experiment, 78 male BALB/c mice without external pathological signs, weighing 16–18 g and 4–6 weeks of age were used. They were divided into the following groups and subgroups:

1. Healthy animals as a control group: subgroup K receiving 50 µl of saline orally during 3 days, withdrawn from the experiment on day 4 (n = 6), and subgroup 2K receiving saline orally following the same regimen, withdrawn from the experiment on day 14 (n = 6).

2. Healthy animals receiving oral saponin for 3 days at a concentration of 5 mg/ml (dose 200 µg/mouse/day): subgroup KS, withdrawn from the experiment on day 4 (n = 11), and subgroup 2KS, receiving saponin according to the same regimen, withdrawn from the experiment on day 14 (n = 11).

3. Animals infected intranasally with influenza A/WSN/1/33(H1N1) virus without correction: subgroup V, withdrawn from the experiment on day 4 (n = 11), and subgroup 2V, withdrawn from the experiment on day 14 (n = 11).

4. Animals infected intranasally with influenza A/WSN/1/33(H1N1) virus and receiving oral saponin at a concentration of 5 mg/ml (dose 200 µg/mouse/day): subgroup SV, derived from the experiment on day 4 (n = 11), and subgroup 2SV, receiving saponin according to a similar regimen and withdrawn from the experiment on day 14 (n = 11).

Study design

Male mice weighing 16–18 g were infected intranasally with influenza A/WSN/1/33(H1N1) virus under brief ether anesthesia by injecting 50 µl of allantois fluid containing 5 LD_{50} of the virus. Aliquots of a single pool of allantois fluid were used, frozen and stored at a temperature of –20 °C. Clinical symptoms confirming the development of viral infection were progressive weight loss and the development of respiratory failure (rapid breathing over 200 respiratory movements per minute with involvement of the ancillary muscles, cyanosis of the tail, ears and limbs). On the days 4 and 14 after infection, the animals were withdrawn from the experiment by decapitation using ether anesthesia, then the spleen was removed for further follow-up study.

A therapeutic regimen of saponin administration was used, which has previously been shown to be effective in animals during experimental influenza infection and influenza vaccination [13, 14]. A triterpene glycoside with the formula 3-O-a-L rhamnopyranosyl (1→2)-a-L-arabinopyranoside hederagenin, abbreviated as saponin tauroside Sx1, isolated at the Department of Physical and Analytical Chemistry of the Vernadsky Taurida National University from the Crimean ivy *H. taurica* by Professor V.I. Grishkovets [18, 19], was used in the experiment.

Histological study methods

Spleen tissue was fixed in 10% neutral buffered formalin with subsequent treatment in alcohol with increasing concentration, embedding in paraffin and preparation of 4 µm-thick sections according to generally accepted histological methods [20]. Paraffin sections were stained with hematoxylin and eosin to carry out a qualitative assessment of morphological transformations followed by morphometric assessment by tracing the contours of scans of longitudinal and anatomically whole histological sections of the spleen obtained on a Leica scanner with a light pen in Aperio Image Scope morphometric and structural image analysis software (Leica Biosystems, USA). The total area (S, mm^2) and the percentage ratio (relative area) of red and white pulp, as well as the area of lymphoid nodules were determined on the sections. The measurements were taken at a magnification of 200×.

All histological studies were carried out in the Center for Collective Use "Molecular Biology" on the basis of the Central Research Laboratory of the Institute of S.I. Georgievsky Medical Academy of the V.I. Vernadsky Crimean Federal University. The research was carried out with the financial support of the Ministry of Science and Higher Education of the Russian Federation, Priority-2030 program No. 075-15-2021-1323.

Ethical review

The study was approved by the Ethics Committee of the V.I. Vernadsky Crimean Federal University (Protocol No. 10 dated November 23, 2021). The animals were kept in the vivarium on a standard diet with free access to food and water under natural light conditions and care was provided according to the requirements and rules governing the treatment of laboratory animals in accordance with the "Rules for carrying out work using experimental animals" (Order of the Ministry of Higher and Secondary Education No. 724 dated Noovember 13, 1984).

During the study, the principles of the Helsinki Declaration adopted by the General Assembly of the World Medical Association (2000) were observed. The animals were withdrawn from the experiment in accordance with the "International Recommendations (Code of Ethics) for conducting Biomedical Research Involving Animals" of the Council for International Organizations of Medical Sciences (CIOMS) (1985) and the rules of laboratory practice in the Russian Federation (Order of the Ministry of Health of the Russian Federation No. 267 dated June 19, 2003).

Statistical data analysis

Histomorphometric data were processed statistically using the Statistica 10.0 software (StatSoft Inc., USA). The mean

(M), the standard error of the mean (m) and the standard deviation (σ) of the areas of red and white pulp, as well as lymphoid nodules were calculated.

During statistical data processing, the variation series were checked for the normality of the distribution according to the Shapiro – Wilk test between the control and experimental groups of laboratory animals. A Student's t-test confidence interval was used to assess differences in qualitative characters. The differences were considered statistically significant at $p < 0.05$.

RESULTS

Most of the spleen parenchyma of control mice was represented by a red pulp consisting of venous sinuses and a net of reticular strands, in the loops of which there are blood cells counts. The white pulp was defined as lymphoid nodules and periarterial lymphoid sheaths with pronounced zonal distribution. Small and medium-sized lymphocytes, plasma cells, reticular cells and macrophages were visualised in the periarteriolar lymphocyte sheaths. In the central part, located directly near the arterial wall, the dominant cell population was specific macrophages.

The following zones were identified in the lymph nodes: periarterial, mantle, marginal and germinal center. The eccentrically located periarterial zone was represented by a pool of mature lymphocytes that surround the central arteriole. A germinal centre with lymphoblasts was visualised adjacent to the periarterial zone. The above-described zones were surrounded by a mantle zone, represented mainly by macrophages and in smaller numbers by erythrocytes, and small lymphocytes, and tissue basophils. The marginal zone was separated from the mantle marginal sinus, localised around the periphery of the lymph node and consisted of medium-sized lymphocytes and macrophages (Fig. 1a).

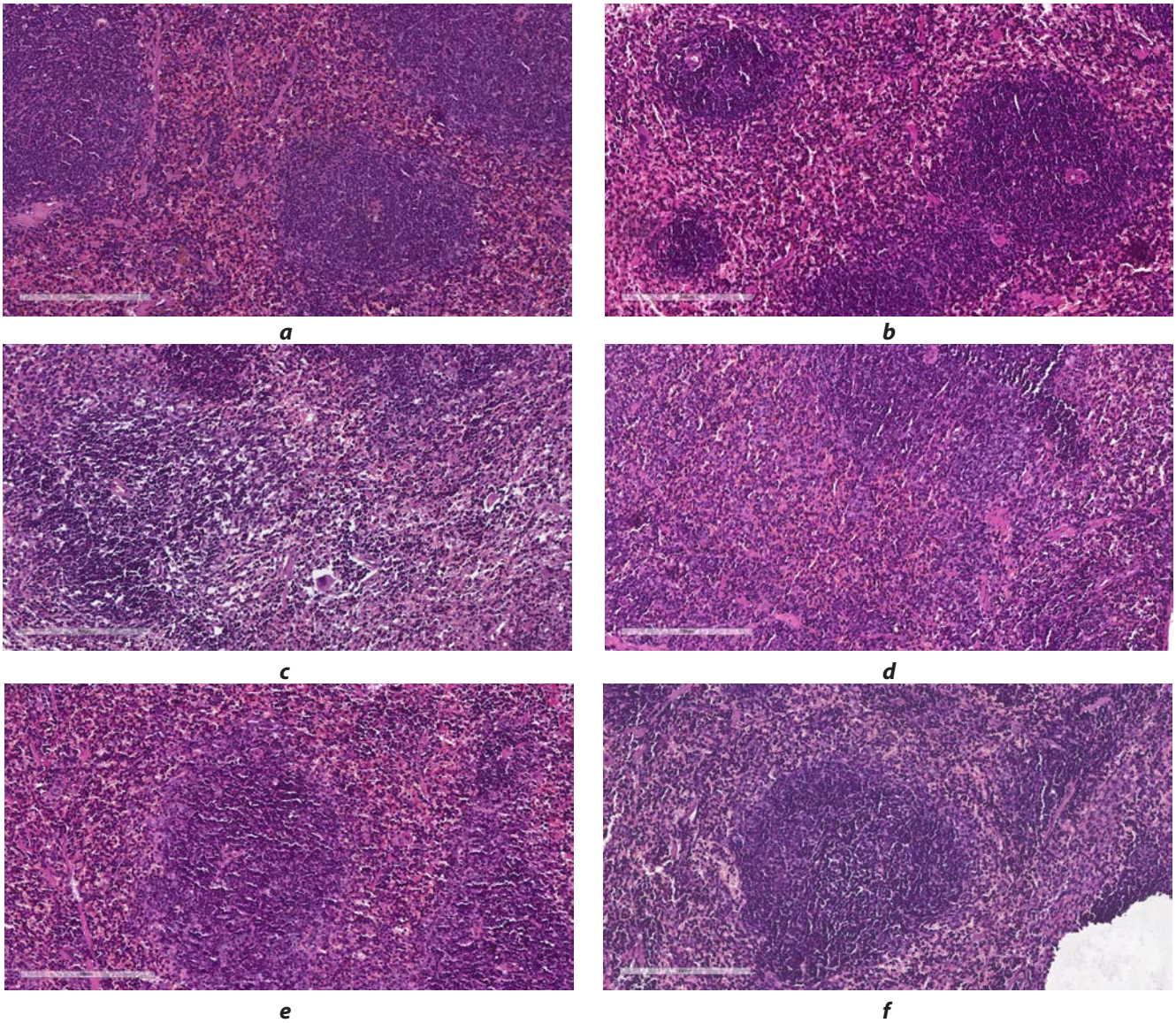

FIG. 1.

*Histological changes in the spleen of BALB/c mice: **a** – control group; **b** – subgroup KS; **c** – subgroup V; **d** – subgroup 2V; **e** – subgroup SV; **f** – subgroup 2SV. Hematoxylin-eosin stain, magnification ×200 Haematoxylin-eosin staining, magn. 200x*

When the histological structure of the spleen tissue was analyzed, the most significant intergroup differences were detected in the K and KS subgroups. In the spleen tissue of the KS subgroup mice a significant hyperplasia of the white pulp in the form of enlarged lymphoid nodules with an increase in the germinal center was observed. Lymphoid nodules occasionally merged with each other and had well-defined zones (Fig. 1b).

Exposure to influenza virus has resulted in significant changes in subgroups V and 2V. In the spleen of mice from subgroup V, there was an increase in the megalokaryocytic reaction, "blurring" of zones and erasing the boundaries of nodules, as well as a decrease in the area occupied by the white pulp. The visible "overgrowth" of the lymphoid nodules was often caused by the fusion of the nearest nodules, which led to a decrease in their total number (Fig. 1b). In subgroup 2V, the histological zones of lymphoid nodules were almost indistinguishable and often there was a complete disappearance of germinal centers, which could indirectly indicate inhibition of lymphocytic proliferation processes (Fig. 1d). Dilated sinusoids often showed hemostasis, neutrophil accumulation, as well as hyperplasia of macrophage cells.

Against the background of drug therapy in the SV and 2SV subgroups, the negative effect of the virus on the spleen of mice was significantly less pronounced. The following histological changes in these subgroups should be noted: despite a significant number of megakaryocytes, a sufficient number of lymphoid nodules, both with and without light germinal centers, were detected in the spleen tissue. Periarterial lymphoid sheaths were visualized, in some of which zoning remained preserved (Fig. 1d, e).

According to morphometric analysis, on the day 4 after saponin administration, as well as against the background of viral infection, a statistically significant increase in the average lymphoid nodule area was observed in all experimental subgroups compared to the control (Table 1). A pronounced statistically significant increase in the mean lymphoid nodules area by a factor of 3.4 has been observed in the healthy animals treated with saponin (KS) compared to the control subgroup (K). In the subgroups of mice V and SV infected with the influenza virus, the increase in the average area of lymphoid nodules was 2.3 and 2.1 times, respectively.

On the 14th day of the experiment, a statistically significant difference in the average lymphoid nodules area between the two subgroups 2K and 2KS was retained, whereby saponin administration more than doubled the lymphoid nodules area. At the same time, in subgroup 2V, a statistically significant decrease in lymphoid nodules area was observed by more than 2.5 times compared to subgroup 2KS. However, oral saponin administration on the day 14 statistically significantly increased the area of lymphoid nodules by 2.7 times in the subgroup of 2SV infected animals compared to the subgroup of 2V animals not treated with saponin.

Parameters of lymphoid nodules total area also naturally underwent significant changes. On the day 4, the subgroup of mice treated with oral saponin (KS) showed a statistically significant 3.9-fold increase in total lymphoid nodule area compared to the control subgroup (K) (Table 2). By the 14th day of follow-up, there was a 2.9-fold difference between the 2KS and 2K subgroups. The actual increase in lymphoid nodules total area in the KS and 2KS subgroups against the background of saponin administration amounted to 8.4 and 4.0 % of the total spleen area, respectively, due to a slight reduction in the area of the red pulp.

During the same follow-up period, despite a compensatory increase in total lymphoid nodules area, subgroup V showed a 7.4 % decrease in the percentage of relative

TABLE 1

ABSOLUTE VALUES OF THE SIZE OF LYMPHOID NODULES IN THE SPLEEN OF MICE INFECTED WITH INFLUENZA VIRUS AND TREATED WITH SAPONIN AT DIFFERENT INTERVALS OF THE EXPERIMENT

The average area (mm^2) of the lymphoid nodule on the day 4 of the experiment			
K* (*n* = 6)	**KS (*n* = 11)**	**V (*n* = 11)**	**SV (*n* = 11)**
0.08 ± 0.01	0.3 ± 0.03	0.2 ± 0.01	0.2 ± 0.01
	$p_K < 0.01$	$p_K < 0.01$ $p_1 < 0.01$	$p_K < 0.01$ $p_1 < 0.01$
The average area S of the lymphoid nodule on the day 14 of the experiment			
2K* (*n* = 6)	**2KS (*n* = 11)**	**2V (*n* = 11)**	**2SV (*n* = 11)**
0.08 ± 0.01	0.2 ± 0.01	0.03 ± 0.002	0.08 ± 0.004
	$p_K < 0.01$ $p_3 < 0.01$	$p_K < 0.01$ $p_2 < 0.01$	$p_2 < 0.01$ $p_3 < 0.01$

Note. p_K – statistical significance of the difference between the current group and the control; p_1 – statistical significance of the difference between the current group and KS; p_2 – statistical significance of the difference between the current group and 2KS; p_3 – statistical significance of the difference between the current group and 2V; * – control subgroup for the days 4 and 14 of the experiment.

TABLE 2

RELATIVE AND ABSOLUTE VALUES OF THE SIZES OF THE WHITE AND RED PULP SECTIONS OF THE SPLEEN OF MICE INFECTED WITH THE INFLUENZA VIRUS AND TREATED WITH SAPONIN AT THE DIFFERENT STAGES OF INFECTION

Groups	K* (n = 8)	KS (n = 11)	V (n = 11)	SV (n = 11)
day 4 of follow-up after infection with influenza virus				
Total area of lymphoid nodules, mm^2	1.7 ± 0.01	6.6 ± 0.01 $p_K \le 0.001$	4.4 ± 0.2 $p_K \le 0.001$	3.9 ± 0.2 $p_K \le 0.001$
Relative area of lymphoid nodules, % S of lymphoid nodules from the total S of the spleen	31.3	39.7	23.9	30.2
Total area of red pulp, mm^2	3.7 ± 0.2	9.9 ± 0.6 $p_K \le 0.001$	13.5 ± 0.8 $p_K \le 0.001$	8.8 ± 0.3 $p_K \le 0.001$ $p_1 \le 0.001$
The relative area of the red pulp, % S of the red pulp from the total S of the spleen	68.7	60.3	76.1	68.7
Total area of the spleen, mm^2	5.4 ± 0.2	16.7 ± 0.4	18.2 ± 0.4	12.8 ± 0.5
day 14 of follow-up after infection with influenza virus				
	2K* (n = 8)	**2KS (n = 11)**	**2V (n = 11)**	**2SV (n = 11)**
Total area of lymphoid nodules, mm^2	1.7 ± 0.01	4.9 ± 0.3 $p_K \le 0.001$	0.7 ± 0.03 $p_K \le 0.001$ $p_1 \le 0.001$	1.4 ± 0.02 $p_2 \le 0.01$ $p_3 \le 0.001$
Relative area of lymphoid nodules, %	31.3	35.3	9.8	18.6
Area of red pulp, mm^2	3.7 ± 0.2	8.9 ± 0.5 $p_K < 0.001$	6.9 ± 0.2 $p_K < 0.001$ $p_1 < 0.001$	6.2 ± 0.06 $p_k < 0.001$ $p_2 < 0.01$ $p_3 \le 0.05$
Relative area of red pulp, %	68.7	64.7	90.1	81.4
Total area of the spleen, mm^2	5.4 ± 0.2	13.8 ± 0.4	7.6 ± 0.02	7.6 ± 0.08

Note. p_K – statistical significance of the difference between the subgroup and the control; p_1 – statistical significance of the difference with subgroup V; p_2 – statistical significance of the difference compared to the subgroup SV; p_3 – statistical significance of the difference compared to subgroup 2V; * – control subgroup for the days 4 and 14 of the experiment.

lymphoid nodules area compared to the control subgroup. On the day 4 of the experiment, there is a statistically significant increase in the area of red pulp in subgroup V compared to the control by 3.6 times, which corresponds to an increase in the relative area by 7.4 %. The introduction of a corrector in the SV subgroup led to a statistically insignificant reduction in the total area of the white pulp by 8.8 % compared to subgroup V against the background of a statistically significant reduction in the area of the red pulp by 53.8 % during these experimental periods.

In subgroup 2V, there was a statistically significant reduction in the total area of lymphoid nodules by 16.4 % compared to the control, which was accompanied by a significant 21.4 % increase in red pulp area. From the day 4 to the 14 of the course of influenza infection, the total area of lymphoid nodules decreased by 5.8 times – from 23.9 to 9.8 %, respectively.

On the day 14 after the commencement of the experiment a statistically significant difference in the total area of lymphoid nodules was detected between the subgroups of infected mice receiving saponin (2SV) and those not receiving the substance (2V). The introduction of a corrector against the background of a viral infection contributed to a statistically significant increase in the total area of the lymphoid nodules by almost 2-fold and in the relative area by 8.8 % compared to the 2V subgroup.

DISCUSSION

Currently, infectious and particularly viral diseases have a significant impact both on morbidity and mortality patterns. In addition, the incidence of influenza, as well as other acute respiratory infections, is the cause of up to 50 % of all cases of temporary disability. The leading cause

of the high pathogenicity of influenza virus is the effect of its haemagglutinin superantigen proteins and NS1 and NS2 non-structural proteins that affect both central and peripheral immune system organs [21].

The reduced and inadequate immune status of the infected mice during this experiment is evidenced by the progressive marked depletion of lymphoid tissue in the largest organ of the peripheral part of the immune system, the spleen.

The immunological activity of the spleen is primarily reflected in the number and size of the lymphoid nodules. The periarterial zone of lymphoid nodules is known to be predominantly occupied by T-lymphocytes that form the cellular branch of immunity, whereas the marginal zone of lymphoid nodules is predominantly occupied by B-lymphocytes [22, 23].

The effect of the influenza virus on mice led to the emptying of lymphoid nodules and, consequently, to a decrease in the relative area of the white pulp of the spleen. At the same time, the total area of the organ increased mainly due to hyperaemia and swelling of the red pulp, whose elements could compensatively phagocytize the damaged red blood cells. On the day 4 after infection, there was a decrease in the germinal zone of the follicles, followed by progressive depletion of lymphoid tissue. By the day 14 of the infectious process, the germinative centers in lymphoid nodules, their boundaries, as well as their T-dependent periarterial zones were practically not visualized in most mice. This indicated the suppression of both the humoral and cellular branches of immunity.

Depletion of lymphoid tissue in the spleen of infected mice of both experimental groups could additionally be caused by lymphocyte apoptosis and a reduced rate of lymphoblast proliferation in response to influenza virus exposure, but the extent of programmed cell death was apparently lower in the group with therapeutic tauroside Sx1 administration at all follow-up periods. Morphometrically, a decrease in the size of the mantle and marginal lymphoid nodule zones and expansion of their germinal centres in subgroups SV and 2SV indicate a compensatory increase in the number of less differentiated cells against a decrease in the number of more mature lymphocytes and reduced red pulp hyperemia [23, 24].

Saponins are substances that have long been used in practical medicine due to the presence of a wide range of biological activity. Some types of saponins increase secretion of bronchial glands by stimulating the cough centre, and are therefore widely used as an expectorant. Ivy leaves extracts are the main components of Gedelix, Bronchipret, Prospan (Germany), Herbion (Slovenia) and are often used both for symptomatic treatment of acute respiratory diseases and to relieve symptoms of chronic bronchitis. However, the immunomodulatory effect of saponins has not been practically studied, since many of them have hemolytic activity [25–27].

The increase in lymphoid nodule area caused by the outgrowth of the marginal zone against the background of tauroside Sx1 administration indicates a probable activation of the humoral branch of immunity even in the absence of an infectious agent. Antigen-binding and antibody-synthesising cells are known to be most concentrated in the marginal zone of lymphoid nodules. In addition, a significant number of lymphoid nodules demonstrated lucent centres where B-lymphoblast proliferation was observed. At the same time, we did not detect any signs of the toxic effect of this saponin. The increase in the relative area of lymphoid nodules could also be the result of active lymphocyte migration from central organs of immunogenesis as a result of prolonged exposure to saponin, which may have contributed to the activation of antibody synthesis observed in our previous studies. Oral administration of saponin has previously been demonstrated to potentiate the immunopotentiating effect of intramuscularly administered subunit influenza vaccine. Vaccinated mice that received saponin at a dose of 200 μg per day after each immunisation showed a 2–10-fold increase in virus-specific antibody production against H1, H3 and haemagglutinin of influenza virus type B after 1–3 weeks [13].

From this fact, it can be concluded that oral administration of saponin tauroside Sx1 positively affects the morphofunctional transformations of the spleen (hyperplasia of the white pulp of the spleen, the appearance of new lymphoid nodules, moderate blood filling of the red pulp), which can be characterised as a quite significant immunostimulating effect.

Considering previous data about the ability of influenza viruses to cause immunosuppression by depleting lymphoid tissue, the use of phytoimmunostimulants in combination with antiviral drugs may contribute to a milder course of the viral infection.

CONCLUSION

Currently, the development and study of new effective domestic means for non-specific prevention of influenza that do not directly affect the virus is very relevant, which would exclude the possibility of resistance formation.

The experiment revealed that infection with the H1N1 influenza virus leads to compensatory activation of the animal's peripheral immune system by increasing the white pulp area as well as fullness of the red pulp, thereby increasing the overall size of the organ. On the 14th day following infection, there was a significant depletion of the white pulp, reflected by a reduction in the overall size of the lymph nodes and a loss of their zoning, which reflected the disappearance of the T- and B-lymphocyte germinal centers in them.

It follows from the data obtained that administration of the saponin tauroside Sx1 positively influences the functional activity of the spleen. This is demonstrated by an increase in the proportion of the white pulp of the spleen as a result of an increase in the size of the lymphoid nodules and their germinal centers. The above-described effects of Sx1 tauroside indicate the future prospects for further study and application of a number of saponins being used as immunomodulatory agents.

Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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Kalfa M.A. – material collection and processing.
Kubyshkin A.V. – material collection and processing.
Rybalko S.Yu. – material collection and processing.
Kirsanova M.A. – material collection and processing; editing of the article.