THE COURSE EFFECT OF THE NAPHTHALAN BATHS ON THE STRUCTURAL CHANGES IN THE LYMPHOID APPARATUS OF THE VAGINAL VESTIBULE OF RATS

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ABSTRACT — Relevance. Lymphoid formations of mucous membranes of hollow organs are considered as indicators, changes in which may indicate the effectiveness of any actions. For this purpose, the work was the identification of dynamic changes in the macromicroscopic parameters of the lymphoid tissue of the vaginal vestibule of rats subjected to the course effects of naphthalan baths.
Material and methods. Mature female Wistar rats exposed to naphthalan baths were used in the experiment. As a control, rats subjected to the course of exposure to fresh water baths were analyzed. After fixing the actual material in neutral formalin and subsequent alcohol wiring at the middle of the anterior, middle and posterior third parts of the vagina, cross sections were made with hematoxylin-eosin and picrofuxin according to van Gieson and Weigert. Obtained during the study of digital data were subjected to statistical processing.
The results of the study. The analysis of the data obtained showed that the exchange rate effect of naphthalan baths does not cause pathological changes in the walls of the rat's vaginal vestibule. The course effect of naphthalan baths leads to an increase in the number of lymphoid tissue in the mucous membrane of the vestibule, activation of lymphocytopoiesis, and a decrease in the level of cellular destruction of lymphoid tissue.

INTRODUCTION

Diverse and frequent diseases of the vulva are an important medico-social problem, with threats to health and quality of life, often reducing its duration (7, 2, 4, 13). It is obvious that the effectiveness of various methods and approaches of surgical and conservative treatment depends on the specifics of the nosological forms, stage of the process, the age of the woman and many other factors. An essential role in therapeutic and preventive measures is given to balneological procedures, including the use of naphthalan baths, the clinical effectiveness of which has been repeatedly proven (17, 6). The therapeutic effect of naphthalan is mainly due to the presence in its composition of polycyclic naphthenic hydrocarbons — derivatives of cyclopentanperhydrophenanthrene, having a quad core (ring) structure (8).

It is believed that such a ring system is present in the composition of various hormones, stearins, bile acids, vitamin D and some other biologically active substances (3). The anti-inflammatory effect of naphthalan oil, including the non-tarred naphthalan, is enhanced by its antiallergic (16) and desensitizing effects (3).

Despite the clinical efficacy of naphthalan, however, in the scientific literature there are no experimental substantiations of their efficacy and safety, which are the basis for the development of new regimens for prevention, treatment and rehabilitation for diseases of the vulva and gynecological profile in general.

This makes it possible to consider lymphoid formations as indicators (biomarkers), changes in which may indicate the safety and effectiveness of any environmental effects (11).

The aim of the work was to identify dynamic changes in macroscopic microscopic indicators of the lymphoid tissue of the vaginal vestibule of rats subjected to the course effects of naphthalan baths.

MATERIAL AND METHODS

The experiment was performed using sexually mature female Wistar rats exposed to naphthalan baths (30 rats), in accordance with the schemes adopted in balneological practice. As a control, rats subjected to the course of exposure to fresh water baths and intact animals (30 rats in each group) were analyzed. Analysis of the vaginal smear confirmed the same state (phase) of the ovarian cycle. The total duration of the course effects of naphthalan and fresh water baths is 20 days each. The duration of each bath was 8–10 minutes. Rats were placed in a bath, with contents of 37–38° C. From the experiment, rats were derived simultaneously (by decapitation, in compliance with all ethical standards). After fixing the actual material in neutral formalin and subsequent alcohol wiring at the middle of the anterior, middle and posterior third parts of the vagina, cross sections were made with hematoxylin-eosin and picrofuxin according to van Gieson and Weigert. For lymphoid tissue of the walls of the vaginal vestibule, the percentage of lymphoid nodules with a center of reproduction was determined (the total set

of lymphoid nodules was taken as 100%), the length, width and area of the section at the lymphoid nodules with and without a center of reproduction; the length, width and area of the reproduction centers themselves, the total number of cells of the lymphoid series (their number per 880 μm^2 section) was also measured as part of the diffuse lymphoid tissue, lymphoid nodules without reproduction centers, in the reproduction centers and the mantle of lymphoid nodules.

The percentage composition of cells of different types of lymphoid formations (different morphogenetic forms of lymphoid tissue) was determined: lymphocytes, plasma cells, macrophages, cells with a mitosis picture, lymphoid cells in a state of degeneration, etc. The significance of differences was determined by the value of p ($p < 0.05$) by the method of confidence intervals by the student's criterion.

The results of the investigation and their discussion. According to our data, there are no pathological changes in the walls of the vaginal vestibule of rats as a result of the course action of naphthalan baths, which may indicate their safety.

According to our data, in the mucous membrane of the vaginal vestibule of rats of the experimental groups, all morphogenetic forms of lymphoid tissue are determined by microanatomical methods — lymphocytes in the integumentary epithelium, diffuse lymphoid tissue, located mainly subepithelially, and the same lymphoid nodules located near the small glands vestibule with and without reproduction centers. Lymphoid formations are always located predominantly near the initial sections of the glands, accompanied by their excretory ducts in the form of a rim of three to five rows of cells of the lymphoid series.

In the stroma of the initial divisions, the cells of the lymphoid series are also located in the form of cords, separating the adjacent initial parts; they are arranged in the form of irregularly shaped fields, which are oriented in the loose fibrous connective tissue of the stroma located between the groups of the initial parts (between the lobules of the gland), which can provide immune control over the secretion processes (11). All lymphoid formations of the walls of the vaginal vestibule (diffuse lymphoid tissue, lymphoid nodules) are dominated by lymphocytes, macrophages, reticular cells, plasma cells and other cells of the lymphoid series are constantly determined.

The quantitative analysis shows the almost complete absence of changes with respect to the control after a course of fresh baths. On the contrary, naphthalan baths lead to the activation of the shaping processes of lymphoid tissue, and, in particular, as a result of their use, the proportion of lymphoid nodules with reproduction centers (table 1), considered as the most functionally mature and differentiated form of lymphoid tissue, indicating active flow processes of local immunity (10).

The percentage of lymphoid nodules with a center of reproduction in the walls of the vaginal vestibule of rats of the experimental group as a result of a course of naphthalan baths, compared with the control, in the walls of the anterior third of the vaginal vestibule is 1.97 times more ($p < 0.05$), its middle third — 1.90 times more ($p < 0.05$), posterior third — 1.77 times more ($p < 0.05$) and for the vaginal vestibule as a whole — 1.86 times more ($p < 0.05$) .

The thickness of centered lymphoid nodules in rats as a result of a course of naphthalan baths, compared with the control, in the walls of the anterior third of the vestibule is 1.59 times more ($p < 0.05$), its middle third is 1.42 times more ($p < 0.05$), the posterior third — 1.36 times more ($p < 0.05$) and for the vaginal vestibule as a whole — 1.44 times more ($p < 0.05$), (Table 2).

The length of lymphoid nodules after naphthalan baths, compared with the control, is 1.64 times greater in the walls of the anterior third of the vaginal vestibule ($p < 0.05$), its middle third is 1.59 times more ($p < 0.05$), the posterior third is 1.52 times more ($p < 0.05$) and for the vaginal vestibule as a whole — 1.57 times more ($p < 0.05$), (Table 3).

The area of lymphoid nodules with a center of reproduction in the walls of the vaginal vestibule of rats after the course effects of the naphthalan baths also increases (Table 4)

This indicator, compared with the control, in the walls of the anterior third of the vestibule is 1.48 times more ($p < 0.05$), its middle third is 1.60 times more ($p < 0.05$), the posterior third is 1.55 times more ($p < 0.05$) and for the vaginal as a whole — 1.54 times more ($p < 0.05$).

The individual minimum and maximum of the proportion of lymphoid nodules with the center of reproduction, their length, width, and area in rats after a course of naphthalan baths throughout the entire body are larger compared to the control.

After a course of naphthalan baths, the thickness of the center of reproduction in the lymphoid nodule (for the vaginal vestibule as a whole), relative to the control, increases 1.7 times ($p < 0.05$), the length of the center of reproduction 1.6 times), its area at the cut is 1.6 times ($p < 0.05$). In relation to the control, after a course of naphthalan baths, the thickness of the lymphoid nodule without a center of reproduction increases 2.1 times ($p < 0.05$), its length — 1.5 times ($p < 0.05$), its area at the cut — 1.5 times ($p < 0.05$). As a result of the course of naphthalan baths in the walls of the vaginal vestibule of rats, the number of cells in the

Table 1. *The proportion of lymphoid nodules with a center of reproduction in the walls of the vaginal vestibule of rats after the course of action naphthalan baths (X±Sx; min-max; in %). For 100% of the total set of lymphoid nodules at the cut*

Nature of the effects	N	Division of the vaginal vestibule			
		Anterior third	Middle third	Posterior third	The vaginal vestibule as a whole
Naftalan baths	30	35,6±0,5 30,1-40,2	39,2±0,4 34,4-43,1	40,1±0,5 35,6-46,2	38,3±0,5 35,6-46,2
Fresh baths	30	19,3±0,4 16,3-23,6	22,1±0,3 16,3-23,6	24,2±0,5 18,2-27,8	21,9±0,5 17,2-28,2
Control	30	18,5±0,3 15,2-22,4	20,6±0,3 15,2-22,4	22,7±0,5 16,2-26,6	20,6±0,5 15,2-26,7

Note: *here and below in the tables n is the number of observations.*

Table 2. *The thickness of the lymphoid nodule with a center of reproduction in the walls of the vaginal vestibule in rats after a course of effect of the naphthalan baths(X±Sx; min-max; μm)*

Nature of the effects	n	Division of the vaginal vestibule			
		Anterior third	Middle third	Posterior third	The vaginal vestibule as a whole
Naftalan baths	30	11,6±0,2 8,3-13,7	11,9±0,3 8,3-14,2	12,8±0,3 10,0-15,4	12,1±0,2 9,2-13,7
Fresh baths	30	7,2±0,2 6,0-10,6	8,5±0,3 5,1-10,6	9,8±0,2 7,1-11,4	8,5±0,3 5,0-10,6
Control	30	7,3±0,2 6,0-9,8	8,4±0,3 5,0-10,6	9,4±0,2 7,1-11,4	8,4±0,3 6,1-11,6

Table 3. *The length of the lymphoid nodule with the center of reproduction in the walls of the vestibule of the vagina in rats after the course effect of the naphthalan bath (X±Sx; min-max; μm)*

Nature of the effects	n	Division of the vaginal vestibule			
		Anterior third	Middle third	Posterior third	The vaginal vestibule as a whole
Naftalan baths	30	13,6±0,3 8,2-15,7	14,9±0,4 8,2-17,3	15,8±0,4 10,0-18,5	14,8±0,4 9,2-17,7
Fresh baths	30	8,2±0,2 6,2-10,5	8,5±0,3 5,2-10,5	10,8±0,3 7,6-13,3	9,2±0,4 5,2-13,3
Control	30	8,3±0,2 6,2-9,7	9,4±0,3 5,2-12,4	10,4±0,3 7,6-12,4	9,4±0.2 6,6-11,8

Table 4. *The area of the lymphoid nodule with the center of reproduction in the walls of the vaginal vestibule of rats after the course effect of the naphthalan baths (X±Sx; min-max; $mm^2 x 10^{-4}$)*

Nature of the effects	n	Division of the vaginal vestibule			
		Anterior third	Middle third	Posterior third	The vaginal vestibule as a whole
Naftalan baths	30	22,5±0,3 18,4-25,2	24,1±0,4 18,4-27,1	25,1±0,4 19,6-28,3	23,9±0,4 18,4-27,1
Fresh baths	30	15,7±0,3 11,0-18,6	16,3±0,3 11,0-18,6	17,1±0,4 12,2-20,3	16,4±0,4 11,0-18,6
Control	30	15,2±0,3 12,2-17,1	15,1±0,3 12,2-17,1	16,2±0,3 12,5-19,7	15,5±0,3 12,2-18,3

lymphoid row also increases. In relation to the control, the value of this indicator in the diffuse lymphoid tissue is 1.4 times higher than the control ($p < 0.05$), in lymphoid nodules without a reproduction center — 1.3 times ($p < 0.05$), in reproduction centers lymphoid nodules and in their mantle zone — 1.4 times ($p < 0.05$). The result of naphthalan baths is an increase in the percentage of lymphocytes (1.1–1.2 times, $p < 0.05$) — one of the most active participants in the processes of immune protection (12). There is an increase in the number of cells of the lymphoid series with signs of mitosis, which indicates the activation of lymphocytopoiesis; the level of cellular destruction in the composition of the lymphoid tissue of the vaginal vestibule of rats is reduced.

Analogous changes in the cellular composition of the lymphoid tissue, as a result of the course action of iodine-bromine and bituminous baths, by the example of the lymphoid apparatus of the larynx of rats previously showed (14). Other authors cite the similar data (15, 1, 5), which indicates the importance of conducting balneological procedures and their effectiveness, which is of significant theoretical and practical importance.

CONCLUSIONS

1. Course effects of naphthalan baths do not cause pathological changes in the walls of the vaginal vestibule of rats, which is proved by morphological methods.
2. As a result of the action of naphthalan baths is the activation of local immune processes (local immunity) of the mucous membrane of the vaginal vestibule of rats.
3. The course effect of the naphthalan baths leads to an increase in the number of lymphoid tissue in the mucous membrane of the vestibule, activation of lymphocytopoiesis, and a decrease in the level of cellular destruction of lymphoid tissue.

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