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Flow phenomena of experimentally induced endometriosis in rats during the immunocorrection with a metabolic immune response modifier 5 amino-1,2,3,4-tetrahydrophthalazine-1,4-dione of sodium salt

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ОСОБЛИВОСТІ ПЕРЕБІГУ ЕКСПЕРИМЕНТАЛЬНО-ІНДУКОВАНОГО ЕНДОМЕТРІОЗУ У ЩУРІВ ПРИ ІМУНОКОРЕКЦІЇ МЕТАБОЛІЧНИМ ІМУНОМОДУЛЯТОРОМ 5 АМІНО-1,2,3,4-ТЕТРАГІДРОФТАЛАЗИН-1,4-ДИОНУ НАТРІЄВОЇ СОЛІ

The results of the experimental observation of the metabolic immune response modifier 5 amino-1,2,3,4-tetrahydrophthalazine-1,4-dione of sodium salt impact on endometriosis genitalis externa in 50 scrub rats are reflected in the article. It is established that immunocorrective therapy held by metabolic immune response modifier in female rats with endometriosis provides a recovery of a sexual cycle and improves the experimental disease procedure.

У статті відображено результати експериментальних спостережень впливу метаболічного імуномодулятора 5 аміно-1,2,3,4-тетрагідрофталазин-1,4-діону натрієвої солі на перебіг зовнішнього генітального ендометріозу в 50 безпорідних щурів. Встановлено, що проведення імунокорективної терапії запропонованим метаболічним імуномодулятором у самок-щурів з ендометріозом забезпечує нормалізацію статевого циклу та покращує перебіг експериментально індукованого захворювання.

Staging of problem and analysis of recent researcher and publications. The recent researches have proved that endometriosis is a gynecological presentation of a system pathology, which is underlied by a dysregulation of different immune and hormonal processes [1–3]. As of today the frontmost theories of pathogenesis of endometriosis are the following: translocation, dysontogenetic, metaplastic, metastatic, dishormonal [4–7]. However, none of the proposed conceptions for development of the illness cannot fully explain its pathogenesis and diversity of endometriosis location. The undeniable fact is that well-functioning immunity system does not allow the endometrium cells to survive beyond the uterine cavity. You may notice that women who suffer from endometriosis have the immunity system dysfunction [8–11]. Immune disorders are followed by ectopic endometrium enlargement, which leads to inflammatory response with macrophage activation. On this basis, it is appropriate and pathogenetically substantiated to provide the adequate immunocorrection [12–14].

One of the modern immune response modifiers is 5 amino-1,2,3,4-tetrahydrophthalazine-1,4-dione of sodium salt. The medicine guarantees the antioxidant

effect and has anti-inflammatory property, which influence positively on the course of the disease. Its main features are caused by the ability to influence the functional and metabolic activity of macrophage [12–14].

The aim of our work was to examine the impact of the metabolic immune response modifier 5 amino-1,2,3,4-tetrahydrophthalazine-1,4-dione of sodium salt on the peculiarities of estrous cycle and the organic symptoms of endometriosis in rats during the experiment.

Materials and methods. The experiment on 50 white female rats was carried out. The scheme of the experimental model of endometriosis included autotransplantation of the left uterine horn fragments to the internal surface of the uterus in such a way that the endometrium was turned to the abdominal cavity.

The investigated animals were divided into two groups: the main group consisted of 25 scrub female rats with experimentally induced endometriosis, which were given hypo of 5 amino-1,2,3,4-tetrahydronaphthalene-1,4-dionesodium salt dosed by 2 mg/kg per day in 1ml in physiological solution during 15

days; the control group included 25 scrub female rats with experimental endometriosis.

The animals were given hypo in equivalent amount of physiological solution during 15 days. The rats of both groups were kept in the standard conditions and were fed with the same food. The medical treatment with the use of proposed immunomodulatory started in 4 weeks after the surgery. The study of the estrous cycle phases duration in both groups started the day after 15-day therapy course.

The course of operation: after the manipulation of the front uterus paries, the layer uterine incision was executed. After the revision of organs of the abdominal cavity, the left uterine horn was knifed and surgical hemostasis was executed, the fragments of endometrium in size of 2 mm, sewed to the internal front surface of the uterus, were selected. In the process of the surgery the polyamide sutures produced by "Olymp" firm (CL UA 24.4-13725905-002:2007).

During the experiment, the researchers observed the experimental animals during the diestrus, proestrus, estrus and metestrus phases. To study the estrous cycle phases in the morning time the sampling of vaginal smears was carried, the samples were colored (azure-II-eosine) according to the method of Romanovskiy. The differentiation of cycle phases was carried after counting of the types of cells and the studying of their correlation.

Research results and their discussios. Before the surgery in the studied groups of animals the length of the estrous cycle was determined, which on average was 4.4 days, an average diestrus (resting phase) lasted for 2.4 days, the average duration of prestrus was 13 hours, the duration of estrus was 1.2 days, the metestrus average duration was 7 hours.

3 weeks after the operation the changes in the length of the estrous cycle phases in both series of studied animals were found. Thus there is a significant increase in the average duration of diestrus to 3.5 days, reducing of the prestrus phase to 10 hours and estrus to 23 hours; the overall length of the estrous cycle increased by 5.2 days.

Such dynamics of sexual cycle time parameters changes in experimental animals series was regarded as a primary manifestation of endometriosis, that was corroborated in future by data evaluation of macroscopic and microscopic examinations of genitalia after the animals withdrawal from the experiment.

After fifteen-day course of the immunotherapy, 5 amino-1,2,3,4-tetrahydrophthalazine-1,4-dione sodium salt, reducing of the general duration of the estrouscycle to 4.6 days and duration of diestrusato 3.1

days in animals of a core group were revealed (before immunotherapy realization, compared to the indicators of the core group's animals, which are affected with endometriosis). It was found, that proestrus normalization phase, the duration of which corresponded to indicators of intact animals (13 hours) and increasing of estrus duration to 26.4 hours, comparing estrous cycle indicators of mentioned animals' group with experimentally induced endometriosis, which were fixed before applying immunotropic medicines.

Deviation of the estrouscycle phases duration of controlled group's animals, which emerged after the surgery, was stable and was not changed before and after fifteen-day course of subcutaneous injection in equivalent amount of solution 0.9 % NaCl.

It should be admitted that the metestrus duration was not changed before and after the surgery in both examined animals' groups, herewith, fifteen-day therapy course in both groups also did not influence the current sexual cycle phase duration.

Three weeks after the surgery, in two observed series of animals during visual observation of the areas of a front abdominal wall and autograft fixed to, it was revealed that the focuses of endometriosis, appeared under the peritoneum surface, hadcyanotic shade. Slight accretion of connective tissue of endometrial tissue was observed.

During the macroscopic evaluating of the endometriosis focuses of controlled animals' group, increasing ectopic implant sizes was revealed in further three months. Herewith, the diameter of the ectopic focuses increased and became 2.6 millimeters after eight weeks. After ten weeks the average diameter of implants was 2.9 millimeters, and after twelve weeks the diameter increased to 3.2 millimeters. During active increasing of endometrioid heterotopias focuses, adhesive process in the abdominal cavity was noted.

Extremely different dynamics of the ectopic implants focuses increasing was observed in core group's animals. Eight weeks after the surgery, the diameter of ectopic focuses did not change and was appropriate to autografts sizes at the moment of surgery (2.0 millimeters). During ten weeks and twelve weeks the diameter of implants was slightly increasing, and formed 2.2 millimeters and 2.4 millimeters in accordance. Adhesive process in abdominal cavity did not emerge in indicated terms.

Discussion of the results. After the experimental surgery, aimed to develop the endometriosis in both groups of experimental animals, it was marked increasing in the development of the overall length of the estrous cycle – by 30 %, diestrus – by 46 % and reduce proestrus phase to 30 %, estrous by 25 %,

compared to the terms that were found in these animals before surgery. Herewith, in the control group these divergences of sexual cycle time parameters were stable and were observed in the future after the 15-day subcutaneous injection of saline.

Another dynamics of the sexual cycle was observed among the animals of the main group, after the therapy with 5 amino-1,2,3,4-tetrahydroftalazyn-1,4-dione sodium salt. The normalization of estrous and proestrus phases was observed in current series of experimental animals. In this group the reduction in the total length of estrous cycle was revealed by 19 % and diestrus cycle – by 13 %, compared with the current indicators of sexual cycle of the control group animals.

Macroscopic evaluation data of pathological changes showed that the therapy with low-molecular weight immunomodulators makes a positive effect. Thus, among the animals of main group after 8 weeks of the surgery the diameter of endometriosis lesions was stable. While among the animals of control group,

during the same period, the diameter of endometriosis lesions was increasing by 30 %.

At the same time, among the animals of the main group, after 8 and 12 weeks after the surgery the diameter of the ectopic lesions was lower by 32 % and 34 % – respectively, compared with the indicators of control group animals.

Conclusion. The treatment of the scrubrads with experiment-evoked endometriosis by metabolic immune response modifier 5 amino-1,2,3,4-tetrahydrophthalazine-1,4-dione sodium salt leads to the reducing of the endometrioidheterotopia. After the course of the immunocorrecting therapy the augmentation of the proestrus and estrus phase is observed to increase and at the same time the diestrus phase contraction is observed to reduce. Thus, the proposed immunotropic medicine relaxes the expression of the sickness organic effect and provides normal sexual cycle.

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