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Mast Cells of Thymus, Adrenal Glands and Liver at the Acute Experimental Inflammation and Its Correction By Preparation NICA-EM.

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ABSTRACT

The conducted research is devoted to investigation of a condition of mast cells system of thymus, liver and adrenal glands of mammals at an acute experimental inflammation under the influence of an immunomodulatory preparation "NICA-EM". It is shown that at the studied model of an inflammation in control group there is a decrease in number of MCs in a thymus and increase in a liver and adrenal glands. Thus the number of the degranulated MCs increases in all studied organs. Preparation "NICA-EM" renders an anti-inflammatory effect, and system of mast cells of an organism of rats treated with the preparation reacts in the universal way. Thus, action of a preparation brings to uncertainly significant decrease in number of MCs in a thymus and liver in comparison with parameters of intact animals, against increase in their number in adrenal glands. The number of the degranulated MCs in comparison with intact animals increases in thymus and adrenal glands, but thus their number is less, than in control. Thus, the conducted research testifies that application of a preparation "NICA-EM" leads to intensification of degranulation of MCs in the studied organs at acute inflammatory reaction against less considerable, than in control, migration of MCs from a thymus.

Keywords. Inflammation, mast cells, immunomodulation, thymus, adrenal gland, liver.

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INTRODUCTION

Mast cells (MCs), or labrocytes, are an object of the continued interest rather long time. It may be explained by polyfunctionality of these cells, and also that they participate practically in all adaptative and pathological processes in an organism of mammals [9]. The mediator of MCs, heparin, provides maintenance of rheological properties of blood and modulation of cell-mediated response at immune and inflammatory processes. Labrocytes are the multipotent cells. They are found in all tissues of mammals except for osseous and cartilaginous.

There are distinguished two subpopulations of mast cells: 1) the mast cells of connecting tissue designated as CTMC (connective tissue mast cell) or MCt (mast cell tripthase); 2) the mast cells of mucous membranes designated as a MMC (mucosal mast cell) or MCtc (mast cell tripthase and chimase). As growth cofactor for the first population serves IL-3, and for the second — IL-3 in combination with IL-4. It is shown that mast cells leave a marrow in an unripe state, accumulate in thymus and finally ripen in other tissues of an organism under the influence of cytokines [45]. MCs show plasticity, mature MCs show extensive proliferation potential [30,40].

These cells form close contacts with a terminals of fibers of vegetative nervous system, and very frequent – with peptidergic ones. An existence of bilateral ties between them is also shown [13, 31, 32, 38].

It is usual to consider mast cells as effectors in IgE-mediated allergic or antiparasitic responses of organism; but in the last two decades researchers have found that labrocytes are also involved in innate immunity and inflammatory processes by releasing a large array of mediators of inflammation [4, 16, 17, 18]. These mediators include such compounds as histamine and MC's specific proteases previously stored in cytoplasmic secretory granules, newly synthesized lipid mediators such as leukotrienes or prostaglandins and also a variety of cytokines, chemokines, and growth factors.

The idea that mast cells are involved in the processes of initiation and sustaining of autoimmunity is based on considerable data from studies of both experimental animal models and human diseases [13, 14, 42, 43].

The research about the roles of mast cells in immune and autoimmune diseases remains object of an extensive discussion. The further studies of peculiarities of these interrelations are important for creating new MCs-targeted therapies [7].

The important role and participation of MC in development of a number of autoimmune diseases is shown [46], in particular, in case of rheumatoid arthritis [6,39], type I diabetes [14,28,35], chronic urticaria [5,8].

Mast cells actively participate in adaptational reactions at an inflammation. They are a source of mediators of an inflammation: of a histamin, proteases, prostaglandins, etc. S.H. He proposed a hypothesis of self-stimulation of degranulation of MS in case of development of pathology. Action of various mediators assumes balance between anti-inflammatory and inflammatory effects, and violation of this balance leads to development of an inflammation [15, 22, 23 ,24, 25].

Mast cells respond to many influences with a complex of stereotypical reactions – as change of quantity of cells, release of mediators from granules and decrease in the content of heparin in them. The defining factors in the mechanism of secretion are strength, character and duration of stressogenic influence, and also individual and age features of an organism [12, 44, 37, 10].

It is possible to claim that MC are one of key links of impact on metabolic processes at any forms of adaptation and pathological processes. Change of a state of MC may be considered as the nonspecific response of peripheral division of any system involved in adaptive processes.

According to modern conceptions, reaction of MC to a stress component of an extreme factor has generalized character. Herewith happens an activation of hypothalamo-pituitary-adrenal axis that cause the increased synthesis of adrenal hormones which influence on functions of mast cells [11].

Reaction of MCs is manifested in the form of two main processes – total degranulation or migration from organs where they are formed (marrow) and accumulated (thymus), into the organs and tissues playing the central role in formation of the general adaptation syndrome and possessing the high level of intensity of proliferative, fermentative and metabolic processes (a liver, adrenal glands, a gut, a stomach). Degranulation of MCs is accompanied by release of biologically active agents which effect depends on tissue in which mast cells are localized [26, 27].

Thus, the system of mast cells is universal, its work is shown both on local, and at the general level. Reaction of mast cells defines further the course of such processes as regeneration, change of the microvasculature, restoration of a microenvironment that is important for regulation of homeostasis, both in physiological conditions, and for development of adaptable mechanisms under the influence of stressor.

In the light of the above described, we conducted research of system of mast cells of a thymus, adrenal glands and a liver of white rats at in model of acute inflammation and at correction of an inflammation with preparation "NICA-EM".

Based on the data described above, we, in turn, forecasted a potential influence of immunomodulatory and anti-inflammatory tissue preparation of an embryonic origin "NICA-EM", derived from embryonic tissues of chickens, on mast cells of mammals on rodent model of an experimental acute inflammation at subplantar introduction of carrageenan. Peculiarities of composition of this preparation allowed us to expect it positive influence on immunity and a condition of an organism as a whole.

The preparation "NICA-EM", made of natural raw materials of an embryonic origin, contains a wide range of biologically active agents. Composition of preparation includes organic acids, including significant amounts of free DNA and RNA, vitamins, enzymes, hormones, macro- and microelements. It was revealed by us earlier the certain influence of preparation on morphofunctional condition of a liver of rats and the expressed homeostasis-stabilizing effect at norm and at experimental non-alcoholic steatohepatitis [2].

"NICA-EM" constitutes a tissue preparation which properties are reached due to a complex of the manipulations directed on activation of biochemical processes in embryonic tissues that allows increasing the level of content of biologically active agents in a substratum, including due to formation of biogenous stimulators. The main feature of technology of this biological product is use of method of high-pressure homogenization (HPH). The used technologies characterize a preparation as the low-molecular nanostructured activated composition. The way of producing of the preparation "NICA-EM" passed patent search and is presented to Federal Ser-vice for Intellectual Property (Rospatent) under the request No. 2014139637 of 30.09.2014.

The complex chemical composition and structural features of this preparation allow expecting its positive influence on mechanisms of regulation of metabolic, hormonal, and probably the immune status, and can cause anti-inflammatory and homeostatic effects.

The selection of organs in which MCs were investigated was defined by that the thymus is a central link in system of mast cells, and a liver and adrenal glands are peripheral ones [34]. All these organs participate in the adaptative reactions proceeding under the conditions modelled by us.

MATHERIALS AND METHODS

Animals

Male Wistar Albino rats of body weights ranging from 170 g to 200 g were used in the study. The animals were fed with standard pellet diet and water ad libitum. The rats were group-housed in polypropylene cages with no more than four animals per cage. They were maintained under standard laboratory conditions with natural dark-light cycle and were allowed free access to standard pellet diet and tap water ad libitum. All the experiments were carried out using three groups, each containing 20 animals. All the animal experiments were performed according to the compliance with the EC Directive 86/609/EEC and with the Russian law regulating experiments on animals. After the end of experiment animals were sacrificed in carbon dioxide chamber.

Carrageenan-Induced Hind Paw Edema in Rats

Acute inflammation was produced by injecting 0.1 ml of carrageenan (1% in saline) locally into the plantar aponeurosis of the right hind paw of the rats [1, 47]. First group served as intact control, where no inflammation was induced. This group was used for evaluation of biochemical parameters. At rats of the control group the carrageenan-induced paw edema was modelled. At animals of experimental group the carrageenan-induced paw edema was also modelled, but at the same time these rats were subdermally injected with preparation "NICA-EM" in a dose of 30 mg per 1 kg of body weight. Injections were made twice, for 14 and in 7 days prior to modeling of an inflammation.

Histopathological analysis

Thymus, liver and adrenal glands were taken and fixed in 10% formaldehyde. After several treatments for dehydration in alcohol, sections having 5µm thickness were cut. Mast cells were selectively stained by routine toluidine blue [16, 33].

Mast Cell Density

An overview of mast cell content in the three tested groups of rats was obtained by counting a minimum of 100 mast cell profiles in thymus, liver and adrenal gland. Using 100× magnification all mast cell profiles that fell within a grid area of 1.00 mm^2 but did not touch the right hand and bottom side boundaries were counted and the data expressed as mast cells/mm^2.

Calculation of number of connective tissue cells was carried out with use of a light microscope Nicon Eclipse 80i at lens magnification 100× in 20 fields of vision with the subsequent recalculation on 1 mm^2.

Degree of degranulation was estimated as the percentage ratio of number of the degranulated cells with signs of violation of integrity of a cytoplasmatic membrane to total number of the analyzed cells.

Biochemical analysis

To check the inflammatory stimulus induced by the intervention of carrageenan the blood level of C-reactive protein (CRP) was assayed. The level of CRP was measured by the use of commercial ELISA (eBioscience (Bender MedSystems)).

Statistical analysis

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 11.0 packed program. Data were presented as mean ± standard. Difference between the control and experimental groups was analyzed using Mann-Whitney U test. $P < 0.05$ was considered statistically significant.

RESULTS

Research of the CRP level showed that this parameter increases in control group to 7.62±0.72 mg/l against 2.84±0.11 mg/l at intact animals. The CRP level in blood of experimental group is also higher, than in group of intact animals, but is significantly lower, than in control and makes 3.87±0.30 mg/l (Fig.1).

In organs of intact animals MCs are characterized by various forms, sizes and localization in tissues. Synthetic activity is more intensively expressed in mast cells of a capsule of adrenal glands. In a thymus and a liver the degranulated MCs are found. Degranulation of labrocytes generally has the directed nature of secretion of granules towards a target cell (Fig 2, 3, 4).

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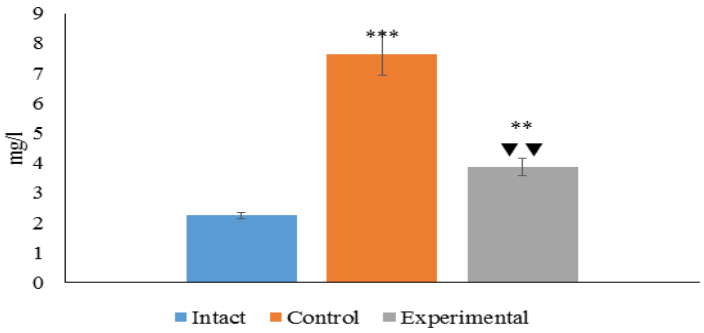

Fig. 1. The level of CRP in blood of rats.

Hereinafter * marks values significantly different from that of the intact (* - p≤0.05, **- p≤0.005,***- p≤0.0005) and▼ marks the significance of differences between control and experimental groups (▼ - p≤0.05, ▼▼- p≤0.005,▼▼▼- p≤0.0005).

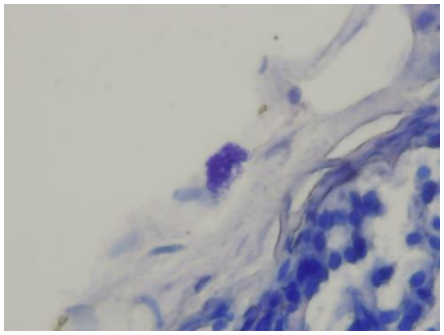

Fig. 2. MC in an adrenal gland capsule of intact rat. Staining with toluidine blue. ×1000.

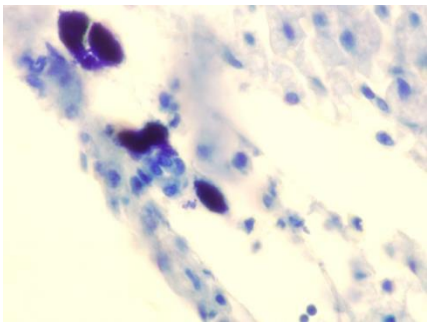

Fig. 3. MCs in thymus of intact rats. Staining with toluidine blue. ×1000.

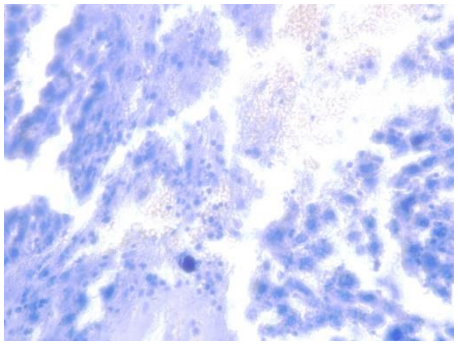

Fig. 4. MCs in liver of intact rats. Staining with toluidine blue. ×400.

Thus the maximal number of MCs per 1 mm^2 is revealed in a thymus and made 170.59±16.69 cells, in adrenal glands it made 39.50±3.88 and 20.0±2.25 in a liver. The coefficient of granulation makes 12.24±1.80% in a thymus, 6.75±1.10% in adrenal glands and 16.05±2.92% in a liver.

In organs of rats of control and experimental groups MCs are also characterized by various forms, sizes and localization. Synthetic activity, as well as in case of intact animals, is more intensively expressed in mast cells of a capsule of adrenal glands. In the studied organs the number of the degranulated MCs considerably increases. Degranulation of labrocytes generally has the directed nature of secretion of granules towards target cells of organs and does not differs essentially from a picture in the studied organs of intact rats.

In a thymus of rats of control group there is an essential decrease in number of MCs to 98.82±8.80 cells per 1 mm^2, but their contents in adrenal glands and a liver increases to 63.46±7.80 and 41.50±6.57 respectively. (Fig 9).

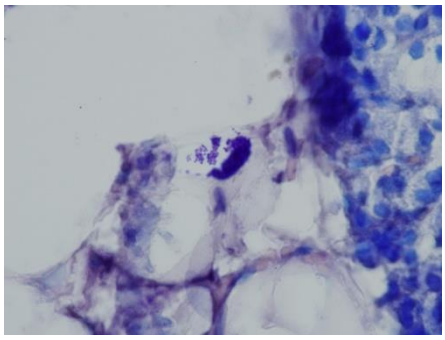

Fig. 6. Degranulated MC in adrenal gland of rat of control group. Staining with toluidine blue. ×1000.

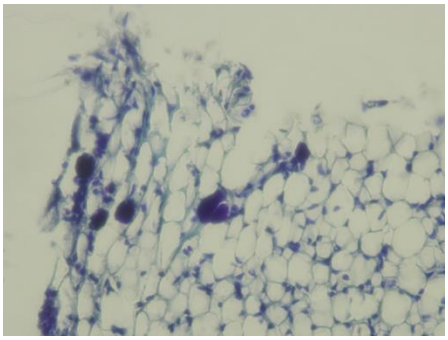

Fig. 7. Degranulated MCs in thymus of rat of control group. Staining with toluidine blue. ×400.

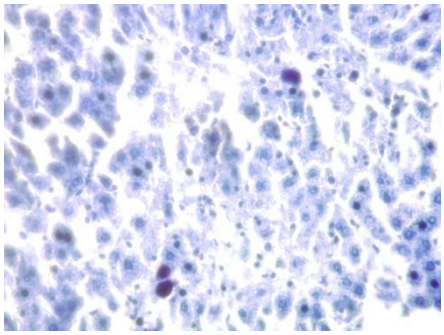

Fig. 8. MCs in liver of rat of control group. Staining with toluidine blue. ×400.

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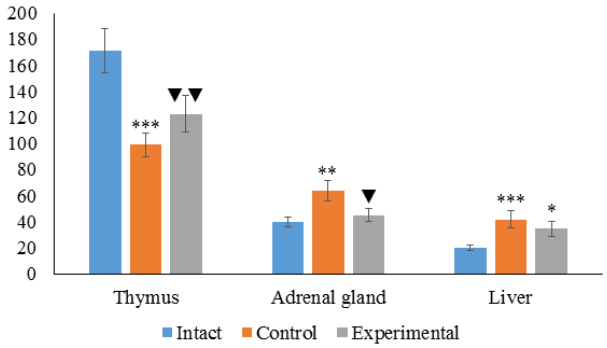

Fig.9. Quantity of MCs in investigated organs of rats.

The coefficient of degranulation in control group increases to 29.87±2.60% in a thymus, 34.0±3.05% in a liver and 18.71±2.28% in adrenal glands. (Fig.13).

At research of organs of rats of experimental group decrease in number of MCs in a thymus to 122.66±13.86 per 1mm^2 is noted. Their number increases in adrenal glands and a liver to 44.90±5.11 and 34.61±5.73 respectively.

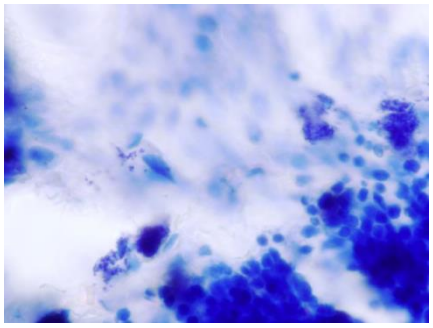

Fig 10. MCs in thymus of rats of experimental group. Staining with toluidine blue. ×1000

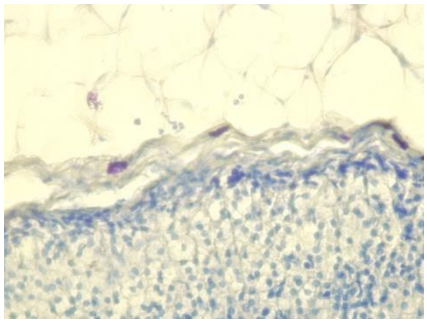

Fig. 11. MCs in adrenal gland of rat of experimental group. Staining with toluidine blue. ×400.

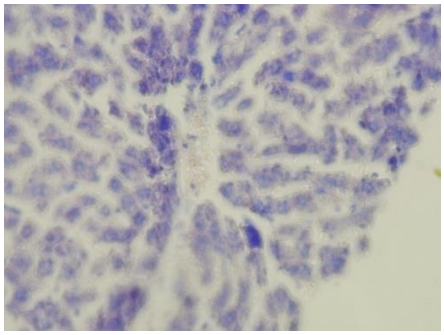

Fig. 12. MCs in liver of rat of experimental group. Staining with toluidine blue. ×400.

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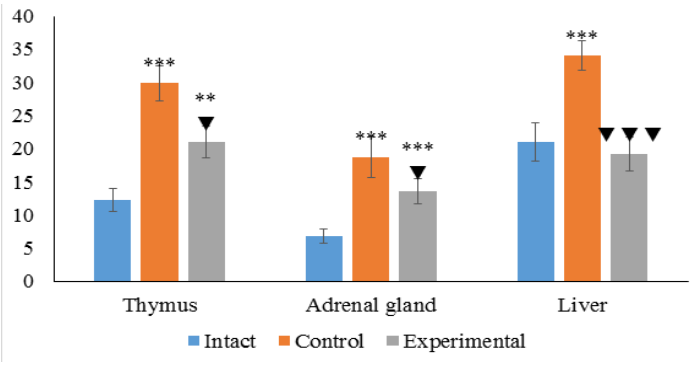

Fig.13. Quantity of degranulated MCs in investigated organs of rats.

As well as in control, there is an increase in quantity of cells in a condition of degranulation. In a thymus the quantity of such MCs among total made 21.01±2.44%, in adrenal glands – 13.58±1.91% and 19.20±2.51% in a liver.

DISCUSSION AND CONCLUSION

As a result of the conducted research in system of mast cells in the studied organs the complex changes having system character are revealed. Mast cells not only play an important role in regulation of physiological functions, but also participate in adaptation to action of extreme factors [36,20].

In particular, at progress of inflammatory reaction the response of mast cells is shown in the organs playing an important role in development of immune reactions (thymus), in the organs defining direct development a stress reaction (adrenal glands) and also in the organs defining maintenance of a homeostasis on the whole (liver). The response of MCs is shown in redistribution of mast cells – decrease of their content in a thymus and increase in adrenal glands and a liver, in connection with migration of these cells, and also in intensification of degranulation of MCs in the studied organs. It is supposed that MCs along with macrophages and endothelial cells play an important role in regulation of function of adrenal cortex, carrying out "setting" of its reaction to a stress [41]. As, as we know, labrocytes extremely rarely proliferate in tissues, processes of increase in quantity of mast cells per unit area after development of inflammatory reaction can be explained with their active migration. Redistribution of mobile cells between tissues may be considered as an important component of an adaptation syndrome [21,29].

The increase in number of mast cells, their polymorphism and number of the degranulated cells is considered as a component of the compensatory and adaptive reaction arising in response to an experimental inflammation.

The CRP level, and also the earlier conducted researches [3] testifies that application of preparation "NICA-EM" renders the expressed anti-inflammatory effect at an experimental model of inflammation. It is possible to assume that the expressed immunomodulatory effect of the tissue preparation "NICA-EM" is based on its ability to correction of the impaired parameters of immune system at all levels: humoral, cellular and nonspecific.

The system of mast cells in an organism of the rats treated with preparation "NICA-EM" reacts in the above described universal way. Thus, action of a preparation leads to uncertainly significant decrease in number of MCs in a thymus and adrenal glands in comparison with parameters of intact animals, against increase in number of MCs in a liver. Thus the number of the degranulated MCs increases in a thymus and adrenal glands in comparison with intact animals, but at the same time their quantity is less, than in control.

Thus, the conducted research testifies that application of a preparation "NICA-EM" leads to intensification of degranulation of MCs in the studied organs at acute inflammatory reaction against less considerable, than in control, migration of MCs from a thymus.

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