

## THE PHARMA INNOVATION - JOURNAL

### Functional status of mast cells with experimental hypoheparinemia

Svetlana Kotyuzhinskaya

1. Odessa National Medical University, Odessa, Ukraine.  
Email: [sveta67kot@mail.ru](mailto:sveta67kot@mail.ru)

**Author for correspondence:** Svetlana Kotyuzhinskaya, Email: [sveta67kot@mail.ru](mailto:sveta67kot@mail.ru)

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We have studied the characteristics of the functional state of mast cells in animals with experimental hyperlipidemia under hypoheparinemia. Stated that the chronic introduction of the blocker heparin animals showed an increase in activity of mast cells accompanied by increased total degranulation of cells. This, in our opinion, cause disorder of the lipid transport system as a whole, with the formation of structural and functional abnormalities in the vessel wall atherogenic direction. The animals that were only on atherogenic diet, observed a sharp increase in the number of mast cells and their secretory activity on the background reduction in the number of mature cells, which may indicate a compensatory mechanism by the lipid-transport system at the cellular level.

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**Keyword:** mast cells, heparin, atherogenic diet.

#### 1. Introduction

According to some researchers, the initiating factor in the formation of atherosclerotic disorders in the body is endothelial dysfunction, which is manifested by increased permeability and adhesiveness, increase secretion of procoagulant and vasoconstrictor factors <sup>[1, 2]</sup>. Of particular interest are evidence that mast cells as a source of endogenous heparin normally protects the arterial wall from arteriosclerosis, and when they lose this ability, the arteriosclerotic changes in the endothelium of accelerated <sup>[3, 4]</sup>. According to the research of several authors stimulation of mast cell degranulation in the presence of rats is accompanied by increased LDL modifications followed their massive uptake by macrophages and foam cell formation that has much importance in the development of atherosclerosis <sup>[5, 6]</sup>. On the other hand, heparin is the major lipoprotein lipase activator, and thereby provides a splitting of

lipoproteins in tissues at an appropriate level to the needs of the organism <sup>[7, 8]</sup>.

Currently, researchers are increasingly of the opinion that in inflammatory and allergic reactions, as well as other pathological processes, significantly alter functionality population of mast cells<sup>9</sup>. Reasons for the differences functioning mast cells under physiological and pathogenic processes are not established yet.

**The aim of this study** was to evaluate the functional state of mast cells in experimental hyperlipidemia blockade on the background of heparin and their role in the mechanisms of atherosclerosis.

#### 2. Materials and methods

Experiments were carried out in accordance with the provisions of the Convention on Bioethics of the Council of Europe (1997), the Declaration of

Helsinki of the World Medical Association (1996), the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986), the general ethical principles experiments animals adopted by the First National Congress of Ukraine on Bioethics (2001).

Examining the status of mast cell population was carried out in two series of experiments on 65 albino adult male rats, weighing 200-250 g, for 21 days. Hyperlipidemia in the experiment was reproduced by the method of V.E. Ryzhkov and V.G. Makarov atherogenic diet containing 30% sunflower oil, 2.5% cholesterol and 0.12% methyluracil [10]. Animals I group (n=25) were kept on atherogenic diet, the rate of load of 1 g fat per 100 g of body weight. Group II animals (n=25) on the background of alimentary atherogenic diet was administered intramuscularly daily protamine sulfate (Protamine sulphate) firm "INDAR" (Ukraine) at a dose of 1 mg/100 g body weight, 2 times a day (by A.M. Ulyanov, Yu.A. Tarasov) [11]. Control animals (n =15) received 0.9% sodium chloride solution in a volume dose of the experimental groups and were on a standard diet, with free access to food and water (minutes of the meeting of the Commission on Bioethics Odessa National Medical University at number 50-v on the 2013.11.22).

Upon expiration of the experiment the animals were withdrawn from the experiment under ether

anesthesia. Object morphological studies of rats served as the mesentery. To assess the activity of mast cells morphofunctional Membrane preparations were prepared by standard methods and stained with toluidine blue (0.1% aqueous solution, pH 2.0). Studies of preparations light-optical microscope were performed on an MBS-6 at a magnification of 400 times. Counting the number of mast cells and the determination of their secretory activity carried out by the usual method D.P. Lindner et al. (1980).

Statistical analysis of the results of research carried out by conventional methods in experimental medicine using of the software package "Microsoft Excel-2000". The results were processed by parametric methods of variation statistics and presented as arithmetic means and the error of the mean ( $M \pm m$ ). The significance of differences between the mean values in the groups was determined by Student's t-test, estimating the probability of the results at a significance level of at least 95% ( $p < 0.05$ ).

### 3. Results and discussion

Analysis of subpopulations of mast cells (MC) in animals reveals the predominance of intact cells with a strong group (type A) and moderate saturation granulation (type B), while the level of cells with a low degree (type C) was below 1.3 times (table 1).

**Table 1:** The qualitative composition of mast cell population in the studied groups, % ( $M \pm m$ )

Group research	type A	type B	type C	type D
Control	33.54±3.27	32.45±2.48	26.53±2.17	7.48±1.33
group I	42.46±3.15*	30.98±2.71	19.63±1.92*	6.93±1.48
group II	18.69±2.09* **	30.37±2.52	31.06±2.19* **	19.88±2.46* **

Note: \* -  $p < 0,05$  - significance of differences with the control group;

\*\* -  $p < 0,05$  - significance of differences with the group I.

Minimum share of a subpopulation of cells were type A (with a very low degree of saturation). This was reflected in the ratio of MC saturation index, which was  $1.94 \pm 0,54$  c. un. Group in intact animals.

Index degranulation in intact animals was  $1.23 \pm 0.06$  c. un. In a population of mostly MC met with mild to moderate degree of degranulation. When atherogenic diet animals, we have not noted marked morphological rearrangements population MC type B and D, however, there was a significant increase in the number of cells of A in 1.27 times

in the background simultaneously reducing the population of cells of type C by 16.39% as compared to intact animals. Proportion of cells varying degrees of degranulation processes showed active synthesis and accumulation of heparin (Table 2). Cell count

with inactive degree degranulation increased 1.42 times, compared to the intact animals, while the number of cells with weak, moderate and strong degree of degranulation was comparable with controls.

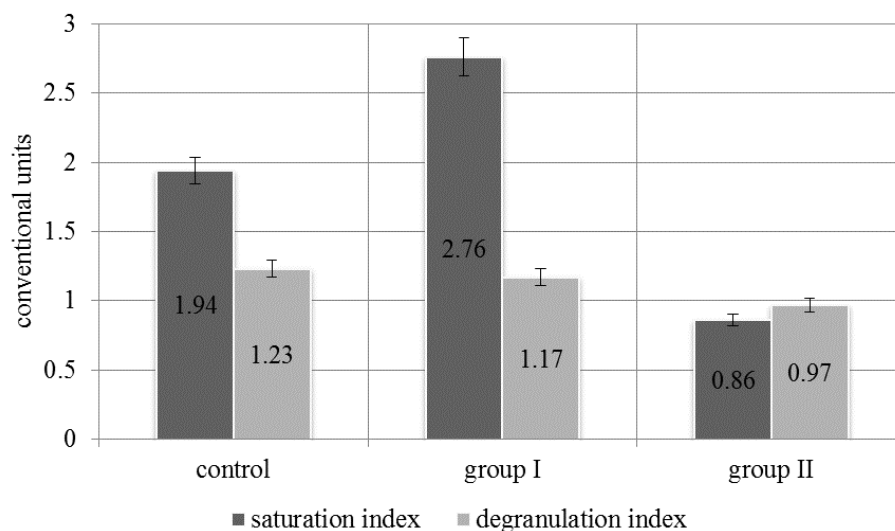
**Table 2:** Ratio of mast cells in the population depending on the degree of degranulation, % (M±m)

Group research	The degree of degranulation			
	0	1	2	3
Control	18.86±1.36	45.82±2.43	23.75±1.57	11.57±0.67
Group I	26.73±1.27*	42.69±1.75	20.15±1.13	10.43±0.54
Group II	37.11±2.43* **	27.44±1.38* **	20.37±1.24	15.08±0.17* **

Note: \* - p < 0,05 - significance of differences with the control group;  
 \*\* - p < 0,05 - significance of differences with the group I.

Confirmation of active synthesis of biologically active amines was saturation index, which is increased by 142.27% compared with controls.

The index of degranulation was comparable with the values of the control group (Figure).



**Fig 1:** Index ratio of saturation and degranulation of mast cell in experimental models.

In the group of animals that were on the atherogenic diet with the introduction of protamine sulfate, we have identified MC, large, rounded with a large homogeneous core, painted pale cytoplasm containing single small dark grains. Are arranged mainly around the vessel and were collected in groups of 2-3 pieces, wherein

these groups are located at a great distance from each other.

Quantitative evaluation of cells showed a decrease in the number of mast cells with a strong degree of saturation on the granular 44.28% and an increase in the number of cells with a very low degree of saturation is more than 2.5 times compared with the control. Simultaneously, there

was a significant increase in the population of cells with a low degree of saturation. Saturation index with decreased by 35.71% compared with controls. Changes in mast cell degranulation in the degree of population led to a decrease in the index of 1.27 times and degranulation. Titer increases significantly with total MC degranulation and simultaneously decreases by more than 1.5 times the number of MC with a weak degree of degranulation compared to control animals. Noteworthy a significant increase in the number of inactive cells in 1.96 times in relation to the control group.

#### 4. Conclusions

As shown by our study, when the content of animals on an atherogenic diet increases dramatically as the number of mast cells and their secretory activity. Such a situation can be regarded as adaptive because of the conditions occurring in a massive secretion of tryptase and / or chymase-heparin complex of the pellets contributes to the binding of LDL, and thereby reduces the formation of atherogenic plaques processes. Increased degranulation of mast cells and their functional activity against the background of a decrease in the number of mature cells may be indicative of compensatory mechanisms at the cellular level. Amplification secretory activity of mast cells and hence an increase in titer of heparin in plasma, with subsequent activation of lipoprotein lipase, it may be one of the factors, which do not allow an adequate model of atherosclerosis in rats.

Contents rats an atherogenic diet with additional administration of protamine sulfate resulted in the decrease in the number of mast cells and alter their structural and functional characteristics. Showed an increase in activity of mast cells accompanied by increased total cell degranulation, which, in our opinion, resulted in a change of the vascular wall in the form of accumulation of lipids in the cells of the intima and copper, changing the elastic membrane and endothelial cells. It should be noted that the synthesis of biologically active substances and degranulation processes are energy intensive, so lack of oxygen leads to inhibition of the function of mast cells, and as a result - a reduction of adaptation options.

Thus, the depletion of mast cells with a consequent reduction of concentration of heparin in the blood plasma lipid-transport disorder will occur in the whole system, with the formation of the structural and functional disorders in the vascular wall atherogenic orientation. It allows us to talk about the new players in the pathogenesis of atherosclerosis.

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