Sexual Dimorphism of Mast Cells in Red Bone Marrow in Normal Rats and Rats Treated with Preparation Endorfain

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We studied immunological effects of Endorfain preparation. Daily oral administration of the preparation stimulated the immune system increasing migration and proliferation and accelerating differentiation of mast cells in the red bone marrow of rats with the pronounced sexual dimorphism.

Key Words: *red bone marrow; mast cells; Endorfain; sexual dimorphism*

Sexual dimorphism is manifested not only at the organ, but also at the cellular and molecular levels [3,5]. Studies in recent years show that male and female immune systems have certain differences [6]. However, there are no data on sexual dimorphism of mast cells (MC) in the red bone marrow (RBM).

Almost all organs contain MC producing biologically active substances and implement the regulation of homeostasis via endocrine and paracrine mechanisms [9].

Immunomodulatory effects of the preparation Endorfain are based on the fact that phenylalanine, the main component of the preparation (each tablet contains 400 mg), being the "building block" of metabolic reactions, promotes production of adrenomimetics, endorphin, catecholamines and, in our opinion, can cause morphological changes in MC. Lipoprotein is located in the center of MC granule, it binds neurotransmitters (serotonin, catecholamines, histamine), which are then covered by heparin, contain interleukins and neutral proteases [2]. Modified bioamine molecules probably cause changes in the degree of maturation, density of heparin and the degree of degranulation of MC. Hormonal status of males and females significantly affects the morphology of immune organs. However, morphological reaction of MC in the thymus and RBM of males and females to phenylalanine is poorly understood.

Here we studied sexual dimorphism in MC of the thymus and RBM in normal rats and rats treated with Endorfain preparation.

MATERIALS AND METHODS

We studied RBM of outbred of 3-month-old male and female rats (n=24) divided into 6 groups: group 1 comprised intact males (n=4), group 2 included intact females (n=4), groups 3 and 4 females (n=4) and males (n=4) orally received with 80 mg/day Endorfain for 7 days, and groups 5 and 6 females (n=4) and males (n=4) received 80 mg/day Endorfain orally for 14 days.

All manipulations with animals were performed according to the Rules for work with experimental animals. RBM was isolated from the femoral bone. Fresh smears were stained with Unna's polychrome toluidine blue used in histological practice to identify basophilic and metachromatic substances. We employed this method combined with morphometry to study tissue mucopolysaccharides and heparin in MC and the degree of degranulation.

By mucopolysaccharides and heparin patterns, MC were subdivided into the following types: 1) orthochromatic MC with blue granules and immature nonsulfated heparin [8]; 2) β_1-metachromatic MC with violet granules and more sulfated immature heparin; 3)

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β_2-metachromatic MC with reddish violet granules due to maturing sulfated heparin [1]; 4) β_3-metachromatic tissue basophils stained red-violet with almost mature sulfated heparin in granules [7]; 5) γ-metachromatic mastocytes with purple granules containing sulfated mature heparin [1].

Based on the classification of D. P. Lindner [4] and G. Yu. Struchko [7], the following types were distinguished by the degree of degranulation: T0 (densely filled with indistinguishable granules, the nucleus is not visible), T1 (distinguishable granules in the cell, the nucleus is not completely masked), T2 (granules are clearly seen in and around the cell, intact plasma membrane and clearly visible nucleus), and T3 (completely degranulated MC with broken plasma membrane).

Morphometric and quantitative analyzes of MC were performed in 10 fields of view under a Mikmed-5 microscope using Sigma Scan Pro 5 soft. The obtained digital data were treated statistically using Microsoft Office software (Word, Excel). Correlation analysis was performed using Cheddock's scale of correlation coefficients (Table 1).

RESULTS

In RBM of normal rats, β_2-metachromatic cells were seen; their total number was 8 times higher in males; T2 cells predominated in males and T1-cells in females. After oral administration of Endorfain for 7 days, β_2 T3-cells predominated in both males and females. The total number of β_2-metachromatic cells decreased 3-fold in males and increased 2-fold in females. On experimental day 14, there were no changes in color index. The total number of β_2-metachromatic cells tended to decrease in males and to increase in females. The degree of degranulation alone showed sexual dimorphism: MC of T1 type predominated in males and T2-type in females. In RBM of animals of both sexes, single T3-cells were detected. Thus, in RBM of normal male rats the number of MC was greater than in females, but the degree of heparin maturity was the same in both. On day 7, Endorfain increased the number of the cells with maturing heparin both in males and females. Inconsistent morphological responses were recorded on day 14: males exhibited more young, females, maturing MC.

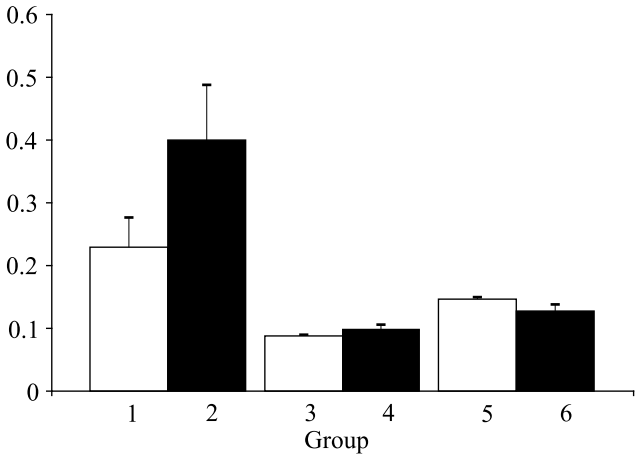

Fig. 1. Changes in the density of MC in RBM of rat females (light bars) and males (dark bars).

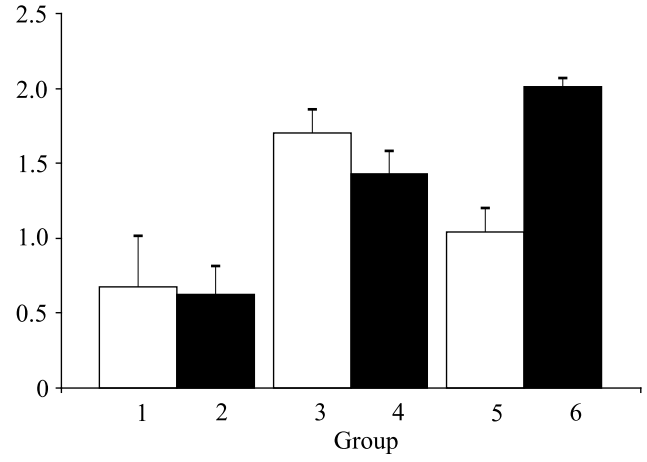

Fig. 2. Changes in the area of MC in RBM of rat females (light bars) and males (dark bars).

Morphometric analysis showed that the density of MC granules in males normally 2-fold surpassed that in females. On experimental day 7, cell density decreased 4-fold in males and 2.6-fold in females. Administration of Endorfain for 2 weeks slightly increased MC density (by 1.3-fold in males and 1.6-fold in females) compared to 7-day administration. At the same time, found no sexual dimorphism on experimental days 7 and 14. Hence, administration of Endorfain for 7 days reduced and for 14 days slightly increased heparin synthesis in males and females (Fig. 1).

Sexual dimorphism in MC area was not revealed in normal rats. Administration of the preparation for 7

TABLE 1. Interpretation of Correlation Coefficients (*r*)

Value	<0.3	0.31-0.50	0.51-0.70	0.71-0.90	0.90-0.99
«+», direct relationship	No	Weak	Moderate	Strong (high)	Very high
«-", inverse relationship	No	Weak	Moderate	Strong (high)	Very high

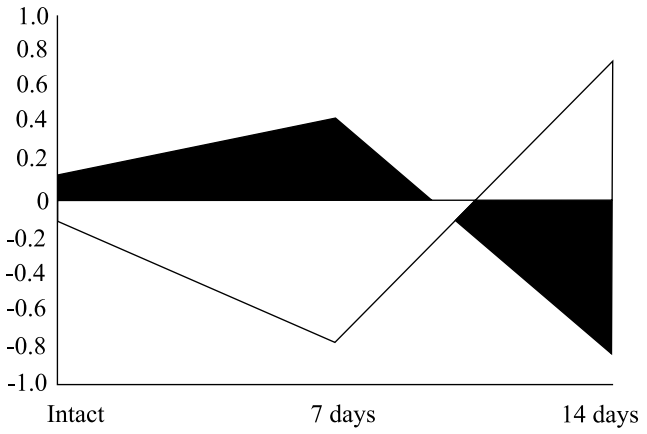

Fig. 3. Changes in correlations between the area and density of MC in rat RBM under the impact of the Endorfain. Light area: males; dark area: females.

days 3-fold increased MC area in males and 2.5-fold in females in comparison with the control. The mean MC area in RBM in females was 1.2 times greater than in males. Administration of the preparation for 14 days 3-fold increased MC area in males and 1.5-fold in females compared to normal rats; it should be noted that MC area in males was 2-fold greater than that in females (Fig. 2).

Analysis of correlations between the density and area of MC in rat RBM revealed no relationship between these parameters in normal male and female rats (Fig. 3). On day 7 of Endorfain administration, a weak direct relationship appeared in males and strong inverse correlation in females. Therefore, the density decreased with increasing of MC area in females due to loosening of the cell content and degranulation. On experimental day 14, a strong direct correlation between the area and density was revealed in females, *i.e*. the increase in cell area was due to heparin accumulation. An opposite pattern was seen in males: strong inverse correlation indicated reduced heparin density associated with increased cell size. Thus, we observed differently directed reaction of MC in RBM of males and females to administration of Endorfain.

Our findings extend our knowledge on sexual dimorphism in immune cells and may be of value for correction of treatment regimens with Endorfain preparation.

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