IMPACT OF REAFERONE ON MICROBICIDAL ACTIVITY OF NEUTROPHILS IN CONDITIONS OF OVARIECTOMY

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Abstract

Purpose: Study of the impact of Reaferone on some peripheral blood parameters, microbicidal activity of polymorphonuclear leukocytes and peritoneal macrophages in ovariectomized rats.

Methods: The ovariectomy was performed under ether anesthesia. Pharmacological, histochemical and immunological methods of research were applied.

Results: Reaferone normalizes the content of leukocytes, erythrocytes, restores activity of oxidative and non-oxidative killing mechanisms of neutrophils and peritoneal macrophages in ovariectomized animals.

Conclusion: Reaferone eliminates negative impact of ovariectomy on some peripheral blood parameters, microbicidal activity of polymorphonuclear leukocytes and peritoneal macrophages.

Introduction

The interconnection and interdependence of nervous, immune and endocrine systems which provide organism's homeostasis, attract our attention more and more [5, 6, 7]. It is proved that an impairment of one of them leads inevitably to a change in the functioning of the whole regulatory neuro-immune-endocrine system [8, 9, 10]. Thus, correction of one of these systems entails changes of another system.

It is well known that physiological or surgical menopause is accompanied by immune suppression, therefore we are interested in studying the efficacy of immunomodulatory drugs in hypoestrogenemia [5, 9].

On the other hand, mononuclear phagocytes and neutrophils not only provide a first line defense against damaging environmental factors, anti-infectious and anti-tumor resistance, immunogenesis processes involved in the neuroendocrine regulation of adaptive responses, but they are also sensitive to the slightest changes in the physiological state of an organism. This fact allows us to use phagocytic reactions as an integral index of the immune system [1, 3].

Objectives

In this respect, the objective of present research is a comparative study of the impact of

Reaferone, which possesses immunomodulatory activity [4], on some peripheral blood parameters, microbicidal activity of polymorphonuclear leukocytes (PMNL) and peritoneal macrophages (PM) in animals with experimental ovariectomy.

Key words: experimental ovariectomy, Reaferone, neutrophils, mononuclear phagocytes, microbicidal activity

Study Area and Methods

In the present study we used 40 white non-inbred adult female rats, weighing 180 - 220 g, kept under standard vivarium conditions. Bilateral ovariectomy was performed under light ether anesthesia. Sham-operated animals got similar surgical access without removing ovaries. The effectiveness of castration was controlled by microscopy of vaginal smears during three weeks. Smears' cell composition remained unchanged during the entire period of observation and corresponded dioestrus phase.

All animals were divided into 4 groups: group 1 - control (intact animals), group 2 – sham-operated (SOVX), group 3 - ovariectomized animals (OVX) and group 4 - ovariectomized animals treated with Reaferone (OVX + R) (interferon alpha-2b) (CJSC "Vector-Medica", Novosibirsk, Russia) in a dose 100 000 IU/kg intramuscularly on the 4th, 7th and 10th days [4], counting the end of the 3-week observation period after the operation as the day zero [6].

The animals were kept under standard vivarium conditions with natural light, standard diet of laboratory animals (All-Union standard GOST R 50258-92), meeting the international recommendations of the European Convention for the Protection of Vertebrate Animals used in experimental studies, along with the regulations of laboratory practice when performing preclinical research in the Russian Federation (State All-Union standard GOST 51000.3-96 and 51000.4-96) and the Order of the Ministry of Health of the Russian Federation of 19.06.2003 №267 "On approval of the regulations of Good Laboratory Practice» (GLP).

Parameters of peripheral blood (number of erythrocytes, hemoglobin level, hematocrit) as well as number of leukocytes in the peripheral blood, microbicidal activity of polymorphonuclear leukocytes (PMNL) and peritoneal macrophages (PM) (against Candida albicans) were assessed in conditions of functioning and blockade of mechanisms of oxidative killing by sodium azide.

The results were recorded the day after the last administration of Reaferone (day 11).

The statistical data processing was performed using methods of variation statistics [2], package Statistica 8.0 software. Checking the normality of data distribution was executed using the Shapiro–Wilk test. The significance of differences was determined by calculating the median and interquartile interval. Analysis of variance was performed applying the Kruskal-Wallis H test, for multiple comparisons Q-criterion was used. The critical level of significance p for statistical criterions was set at 0.05. The data are presented in the text as a percentage of control (intact

animals).

Results

32 days after the surgery we observed decrease in the number of erythrocytes (down to 69.76% relative to control), hemoglobin (down to 91.49%) and hematocrit (down to 74.17%) in ovariectomized animals (Fig. 1A, B, C).

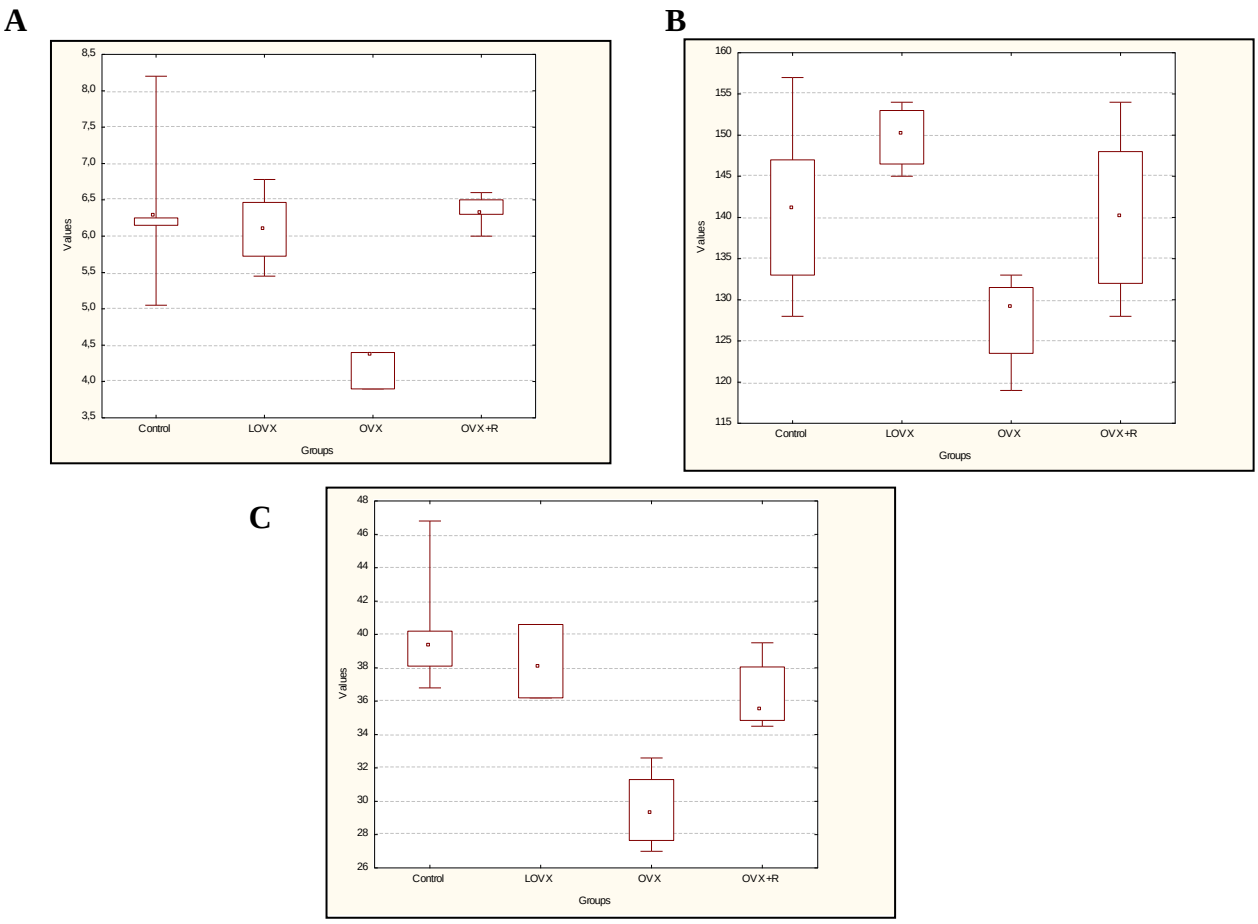

Fig. 1. Effects of Reaferone on levels of erythrocytes (A), hemoglobin (B) and hematocrit (C) in ovariectomized animals

In addition, reduction of the number of peripheral blood leukocytes (for 20% relative to intact and sham-operated animals) was shown.

Content of erythrocytes, hemoglobin, hematocrit and white blood cells were not different in the OVX + R group from those parameters in intact animals (Fig. 1A, B, C).

Increase of colony forming units was revealed in the microbicidal activity assessment of PMNL (up to 139.19%, $p = 0.0086$ and up to 148.65%, $p = 0.0003$, respectively) in the LOVX and OVX groups. This fact demonstrates the suppression of fungicidal oxidative mechanisms of PMNL. As a result, inactivation index of PMNL in the LOVX group was 83.61%, while in the OVX group it reached 79.65%. There was no reduction of fungicidal activity of PMNL (inactivation index was 102.37%) in the LOVX group in conditions of oxygen-dependent microbicidity blockade, whereas significant increase (relative to control ($p = 0.0039$) and sham-operated animals ($p = 0.0016$)) in the

number of colony forming units (up to 136.52% relative to control) was detected in the OVX group. Inactivation index was 69.53% (compared to control), indicating the inhibition of non-oxidative microbicidal mechanisms of PMNL.

In the group of animals treated by Reaferone, fungicidal activity of PMNL did not differ from that of intact animals nor in conditions of functioning neither blockade of oxidative killing mechanisms (inactivation index was 92.37% and 76.92%, respectively).

No descent in fungicidity of peritoneal macrophages was revealed in sham-operated animals. Meanwhile, quite strong suppression of the activity of both oxygen-dependent and oxygen-independent microbicidity factors of mononuclear phagocytes was shown in the OVX group (inactivation index decreased to 61.84% and 59.95%, respectively) (Fig. 2A, B).

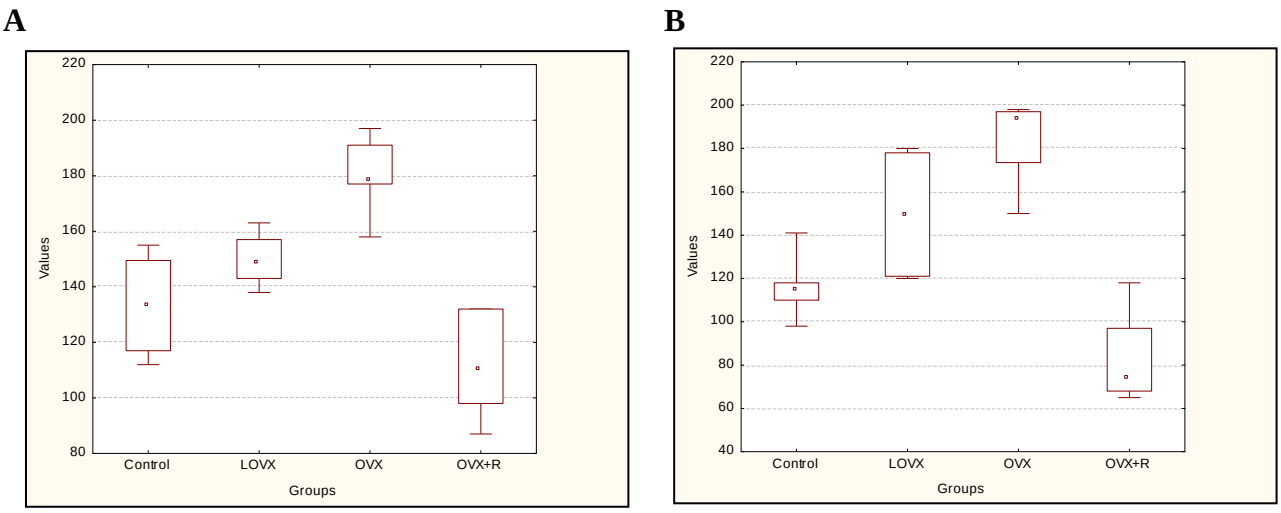

Fig. 2. Impact of Reaferone on microbicidal activity (colony forming units) of macrophages in ovariectomized animals in conditions of functioning (A) and blockade (B) of oxygen-dependent killing mechanisms

Administration of Reaferone completely eliminated oppression of macrophages fungicidal activity in both conditions of functioning and blockade of microbicidal oxidative mechanisms. It was evidenced by statistically significant reduction in the number of colony forming units (down to 82.71% relative to control), with respect to both LOVX and OVX groups ($p = 0.0246$ and $p = 0.0004$, respectively) (Fig. 3A). The amount of colony forming units was 64.04% (relative to control) in conditions of oxygen-dependent killing blockade and it was significant towards the LOVX and OVX groups ($p = 0.0043$ and $p = 0.00005$, respectively) (Fig. 2B).

As a result, application of Reaferone restored the activity of both oxidative and non-oxidative killing mechanisms of macrophages in ovariectomized animals (inactivation index was 119.51% and 120.92%, respectively).

Conclusions

In summary, use of Reaferone in ovariectomized animals contributed an increase of the

number of erythrocytes, leukocytes, as well as a perceptible correction of the functional state of PMNL and peritoneal macrophages to the level of intact animals. We observed full recovery of oxidative and non-oxidative killing mechanisms in neutrophils and macrophages.

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