

# Effect of Carbamylated Darbepoetin Administration at Different Doses on the Thymus and Spleen Structure of Rats

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## Abstract

In this article, the morphological features of the structure of the thymus and spleen under the influence of carbamylated darbepoetin in different doses were studied. The material for the study was 40 white male rats of the Wistar breed, to which the drug was administered 3 times a week subcutaneously in a single dose (50 µg/kg) and twice a dose (100 µg/kg) for a maximum daily therapeutic dose for a man for 92 days, and then for 30 days the animals were under observation. Placebo in an equivalent volume was used as a control substance. The material was processed by standard histological methods, and then stained with hematoxylin-eosin. The results of studies of the drug in different doses showed that the thymus and spleen undergo minor morphological changes compared with the control groups, which is associated with the mechanism of action of the drug.

## Introduction

In connection with the scientific and technological progress in the pharmacological industry, drugs are created and studied that have a wide range of effects on the body. One of them is carbamylated darbepoetin. Relatively recently, in addition to the "classical" hematopoietic activity, this drug has a cytoprotective ("non-classical") activity, which has a protective effect on the cells of the body [1, 2]. Thus, carbamylated darbepoetin inhibits the expression of p53 protein in retinal neurons and induces the expression of endothelial NO synthase in its vessels, as a result of which a protective effect is observed [3]. There is an assumption that the implementation of this activity is mediated by binding to the heterodimeric receptor EPORβCR, which, in turn, leads to a decrease in the sensitivity of cells to hypoxia and activation of anti-apoptotic signalling pathways [4]. At the same time, the effect on the endocrine and immune systems has not been studied enough.

## Purpose

To study the morphological features of the structure of the thymus and spleen under the influence of carbamylated darbepoetin in different doses.

## Methods

The material for the study was 40 white male rats of the Wistar breed weighing 200-220 g, which were divided into 4 groups of 10 animals each. An

experimental animal was administered the drug 3 times a week subcutaneously (under the skin of the back) in a single dose (50 µg / kg) and twice a dose (100 µg / kg) maximum daily therapeutic doses [5] for a person for 92 days, and then for 30 days the animals were kept under control. Placebo in an equivalent volume was used as a control substance. The study was conducted using visual, instrumental and laboratory monitoring of the state of animals [6-11], modern methods of assessing the effect of the drug in accordance with the Scientific Centre for Examination of Medical Products developed by the Federal State Budget Institution Guidelines for conducting preclinical studies of drugs" (2012, volume I), edited by MD A.N. Mironov. After the material was collected, fragments of the thymus and spleen were fixed in a formalin solution, and then subjected to standard histological wiring followed by the manufacture of glass preparations. The latter were stained with haematoxylin and eosin. A microscopic study was performed using an image analyzer consisting of a Nikon Eclipse Ni microscope with a Nikon DS-Fi3 digital camera and Nis-Elements BR 4.60.00 64-bit software.

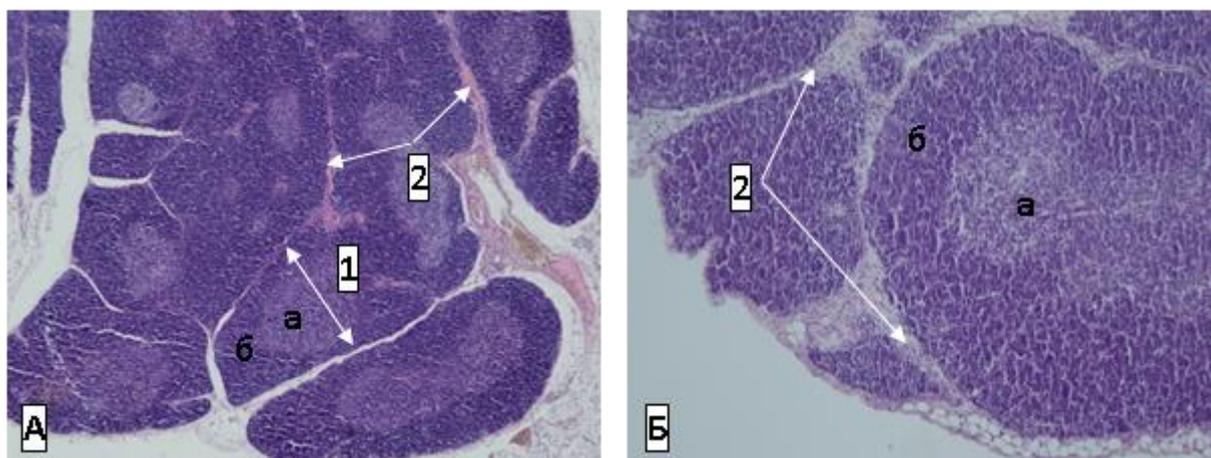
The study was carried out in the preclinical research laboratory of the Center for Pre-Clinical and Clinical Research of the National Research University "BelSU" (308015, Belgorod, Pobeda St., 85), as part of the State Contract with the Ministry of Education and Science of the Russian Federation No. 14.N08.11.0077 from "16 June 2016. The code number" 2016-14-N08-0011-001".

## Main part

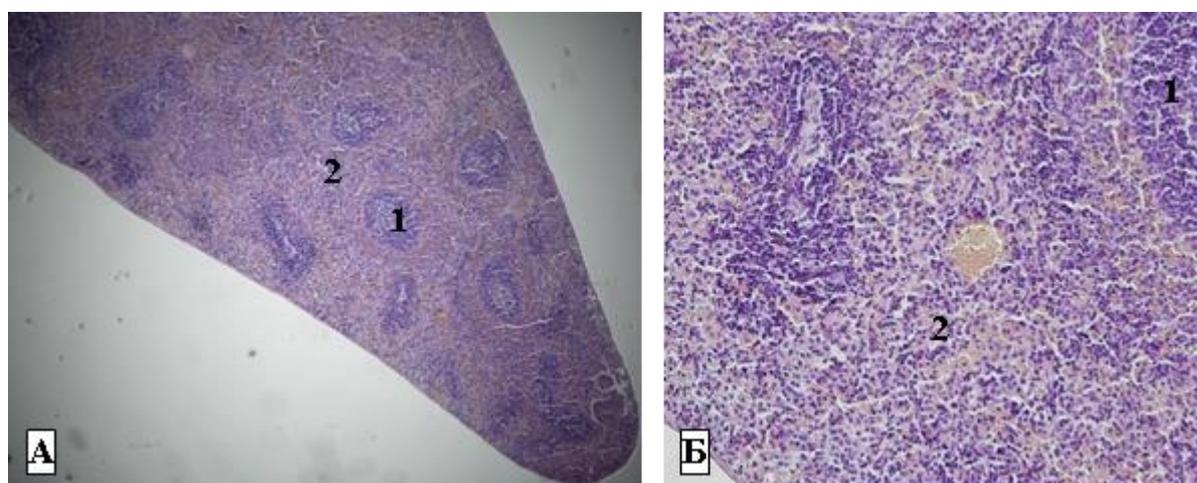
The study of histological preparations of the thymus and spleen of rats after administration of placebo showed that they have a typical structure. After using carbamylated darbepoetin at a dose of 50 µg / kg, the medulla prevailed over cortex visually in the thymus lobules. Connective tissue septae were clearly visualized, in which there were isolated accumulations

of adipose tissue (Fig. 1).

With a similar effect in the spleen clearly visualized lymphatic nodules of small white pulp, located separately from each other. In some of them, germinal centers were identified. The vessels of the red pulp were full. The marginal zone in the nodules was poorly visualized (Fig. 2).



**Figure 1.** Sections of rat thymus after exposure to carbamylated darbepoetin at a dose of 50 µg / kg: 1 — thymus lobule (a — medulla, b — cortex); 2 - connective tissue septae. Staining: hematoxylin-eosin. H. \* 4 (A) and \* 10 (B)



**Figure 2.** Areas of the rat spleen after exposure to carbamylated darbepoetin at a dose of 50 µg / kg: 1 - lymphatic nodules; 2 - red pulp. Staining: hematoxylin-eosin. H. \* 4 (A) and \* 20 (B)

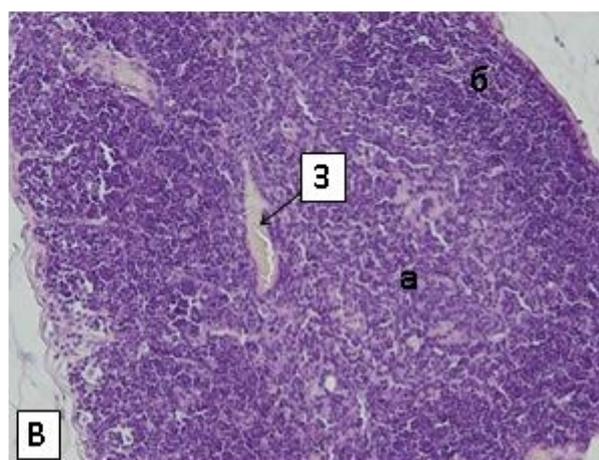
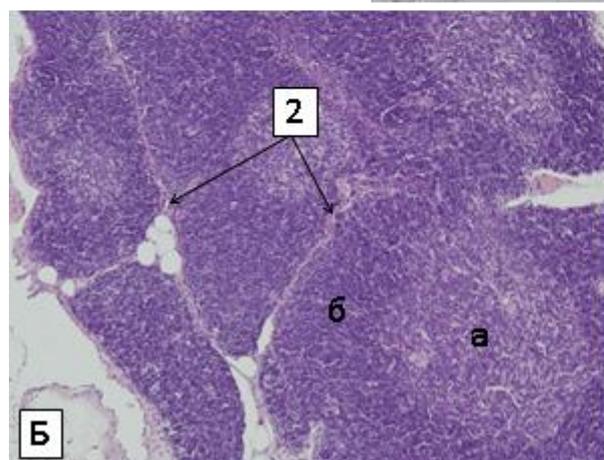
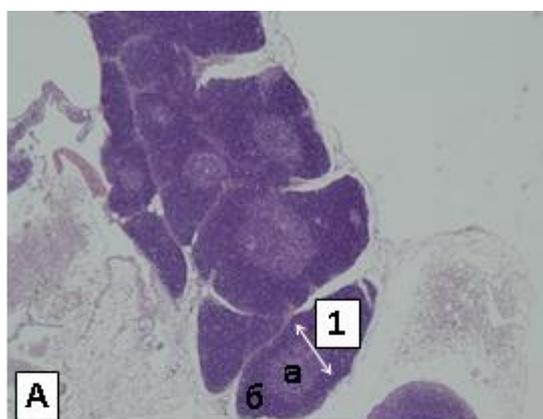
It can be assumed that these features in the structure of the thymus and spleen are associated with the cytoprotective effect of the erythropoietin derivative, which can be implemented in part through its preferential binding to the β receptor subunit (βcR), which starts the process of signal transduction of growth factors and cytokines [12]. And this in turn allows you to actively respond to antigenic material, which is manifested in the presence of clearly defined germinal centers in the lymph nodules of the white pulp of the spleen. This mechanism also affects the processes of differentiation and the eviction of lymphocytes from the thymus into the bloodstream and, as a consequence, is manifested by an increase in the lobules of the medulla compared to cortex.

With a double dose of the drug in thymus sections, the cortex of the lobules visually prevailed over the

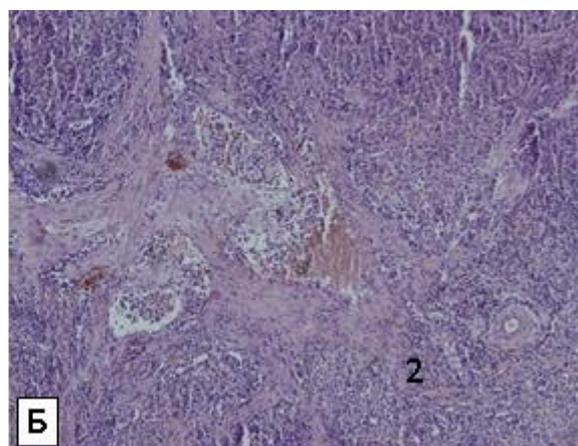
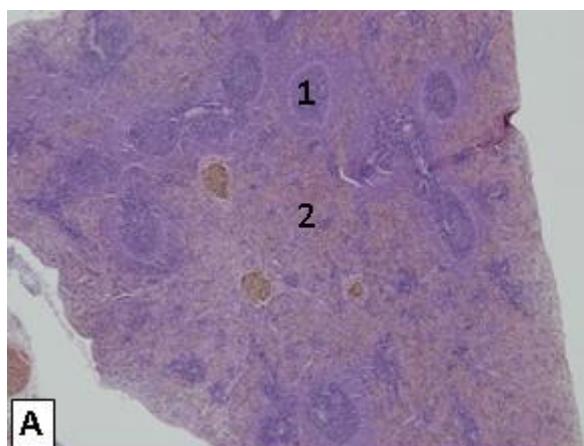
medulla. In the layers of connective tissue between the thymus lobules, isolated adipocyte clusters were found. There were isolated full blood vessels as well (Fig. 3).

In the spleen, the lymph nodules, in most cases, contained germinal centers. They were located in groups, rarely one by one. The vascular network was well expressed with diffuse focal plethora of vessels and capillaries. The marginal zone occupied one third of the lymphatic nodules and often merged with a similar neighbouring nodule (Fig. 4).

These morphological features may be related to the ability of carbamylated darbepoetin to influence the proliferative and antiapoptotic activity of lymphocytes with a double increase in the dose of the drug. So in the thymus lobules the cortex prevailed over the medulla and in the lymphatic nodules of the white pulp of the spleen the marginal zone was developed.



**Figure 3.** Areas of rat thymus after exposure to carbamylated darbepoetin at a dose of 100  $\mu\text{g} / \text{kg}$ : 1 - thymus lobule (a - medulla, b - cortex); 2 - connective tissue septae, 3 - vessel. Staining: hematoxylin-eosin. H. \* 4 (A), \* 10 (B) and \* 20 (C)



**Figure 4.** Areas of the rat spleen after exposure to carbamylated darbepoetin at a dose of 100  $\mu\text{g} / \text{kg}$ : 1 - lymphatic nodules; 2 - red pulp. Staining: hematoxylin-eosin. H. \* 4 (A) and \* 20 (B)

It should be noted that the accumulation of adipocyte groups, clear connective tissue septae in the thymus, as well as the developed vascular network of the spleen are, according to literary data, age-related features of these organs [13, 14, 15].

## Conclusion

Based on the fundamental knowledge of the physiology of intracellular processes, modern pharmacologists are developing tools with universal cytoprotective activity [2, 3, 16]

A preclinical study of carbamylated darbepoetin in different doses showed that the thymus and spleen undergo minor morphological changes compared to the control groups, which is associated with the mechanism of action of the drug (aimed at treating or preventing conditions associated with tissue damage), as well as with age-related changes.

The obtained results allow us to recommend the drug Carbamylated darbepoetin, solution for injections (Pharmapark LLC, Russia) for conducting a clinical study with the purpose of registering the drug in the Russian Federation.

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