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Peculiarities of the influence of cyclophosphamide and imunofan on the processes of apoptosis and necrosis in Peyer's patches of rats' small intestine

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Abstract

In this article, we studied the peculiarities of cyclophosphamide and imunofan effect on the processes of apoptosis and necrosis in Peyer's patches of the rats' small intestine. The study was conducted on 18 white mature rats, which were divided into three groups. Group I was administered imunofan according to the scheme on days 1, 3, 5, 7, 9 of the experiment at a dose of 0.7 µg/kg of body weight of the animal, Group II was administered cyclophosphamide once at a dose of 200 mg/kg (for 1 day), then imunofan according to scheme (on the 2nd, 4th, 6th, 8th, 10th day of the experiment) at a dose of 0.7 µg/kg body weight of the animal, and Group III - intact animals served. Ultrathin sections of Peyer's patches of the rats' small intestine were prepared and studied under electron microscope with further photographing. Mostly one type of cell death - apoptosis is detected in group I animals on the 7th and 30th day of the experiment in Peyer's patches. The number of cells with apoptosis is directly proportional to the observation period, which seems to indicate the ability of imunofan to influence this process, maintaining the constancy of Peyer's patches cellular composition in small intestine. In animals of group II, on the 7th day of the experiment, both apoptosis and necrosis take place, and on the 30th day it is mainly apoptosis. Apparently, this is due to the immunosuppressive effect of cyclophosphamide on the lymphocytes of Peyer's patches of the small intestine, which manifests itself in the early period of the experiment after correction by the immunomodulator.

Keywords: peyers' patches, cyclophosphamide, imunofan, apoptosis, necrosis

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INTRODUCTION

The immune system of small intestine is actively responding to external influences and is on the path of a huge stream of antigenic material. It is characterized by a high rate of renewal of cell populations, in which both the process of apoptosis and necrosis take part (Forchielli and Walker 2005, Proskuryakov et al. 2005, Sepiashvili et al. 2015).

Apoptosis is programmed cell death, which provides control over the cell population in a multicellular organism by balancing the processes of cell proliferation and elimination. Cells with apoptosis are located singly or in small groups and are scattered in the tissues of the organs for which active cell proliferation is characteristic. Apoptosis is an essential condition for maintaining homeostasis of the body. The processes of necrosis affect the entire field of cells, which leads to the shutdown of the function of different sections of tissues and organs (Edinger and Thompson 2004, Savitskaya and Onishchenko 2015).

Immunocorrectors are used in medical practice to maintain the normal functioning of the small intestine immune apparatus. Cyclophosphamide has become widespread among immunosuppressive drugs. It is included in many schemes of antitumor therapy, and is used for the prevention of transplant rejection and treatment of autoimmune diseases (Ahlmann and Hempel 2016, Varga 2007). One of the ways to increase the effectiveness of treatment of oncologic and other diseases with chemotherapeutic drugs is their combination with immunomodulators. The most common of the last generation of chemical drugs, which is used in the treatment of various immunodeficiency states and in oncologic practice, is imunofan (Akbari et al. 2016, Butorov et al. 2007, Mar'in et al. 2009, Özer 2018, Salakhova et al. 2018, Shokri 2016).

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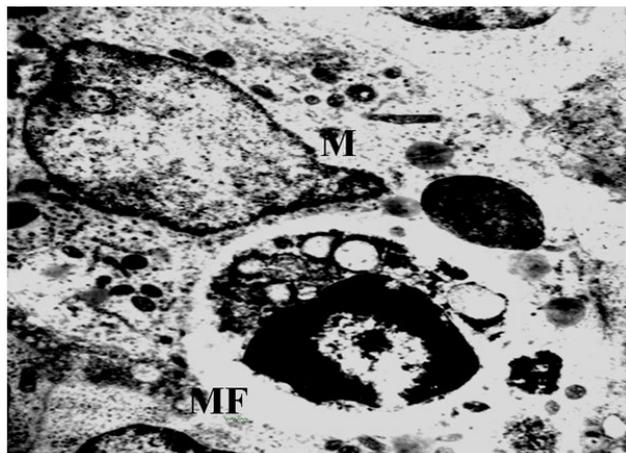


Fig. 1. Peyer's patch on the 7th day of the experiment after exposure of imunofan: M - macrophage, MF - macrophage phagosome, containing a cell at an early stage of apoptosis. Magnification $\times 12,000$

PURPOSE

The purpose of this study was to identify whether immunocorrectors (imunofan and cyclophosphamide) affect cell death in Peyer's patches of reproductive age rats' small intestine.

METHODS

The study was conducted on 18 white mongrel sexually mature male rats weighing 200-250 g. The animals were divided into three groups of 6 each. Group I was administered imunofan according to the scheme on days 1, 3, 5, 7, 9 of the experiment at a dose of $0.7 \mu\text{g} / \text{kg}$ of body weight of the animal, II was administered cyclophosphamide once at a dose of $200 \text{ mg} / \text{kg}$ (for 1 day), then imunofan according to the scheme (on the 2nd, 4th, 6th, 8th, 10th day of the experiment) at a dose of $0.7 \mu\text{g} / \text{kg}$ body weight of the animal, and III - intact animals served.

The maintenance and care of animals was carried out according to the provisions of the European scientific community "The use of animals in research", (2000).

Animals were removed from the experiment on days 7 and 30 after the administration of the drugs. Immediately after extraction of the small intestine, 1 mm^3 volume was isolated from Peyer's patches. Material processing was carried out according to standard methods. Ultrathin sections were prepared on a UMP-4 ultramicrotome of the Sumy IA Elektron, contrasted in a solution of uranyl acetate and lead citrate by E. Reynolds and studied under an EM-125 electron microscope with further photographing.

MAIN PART

On the 7th day of the experiment, single cells with apoptosis were detected in animals of group I in the area of Peyer's patches, as in group III of animals. They are characterized by a decrease in the volume and compaction of the cytoplasm, the cell membrane is not changed. Organelles have a typical structure and are more compact. Chromatin condenses under the nuclear membrane in the form of a definitive demilune. Single cells are also identified in the early stages of apoptosis, which are entirely phagocytised by macrophages (**Fig. 1**).

At this time of observation, in animals of group II, in Peyer's patches of the small intestine, in addition to apoptosis, lymphocytes contain isolated groups of cells with necrosis. Their nucleus decreases in volume, becomes shriveled, dense, chromatin condenses into clumps. In some cells, it is fragmented into basophilic particles or undergoes lysis (**Fig. 2 a, b**).

Mitochondrial swelling and destruction of the organelles are also observed, which causes vacuolization of the cytoplasm. At the same time, enzymes are released from lysosomes and not only the given cell, but also the neighbouring ones are destroyed.

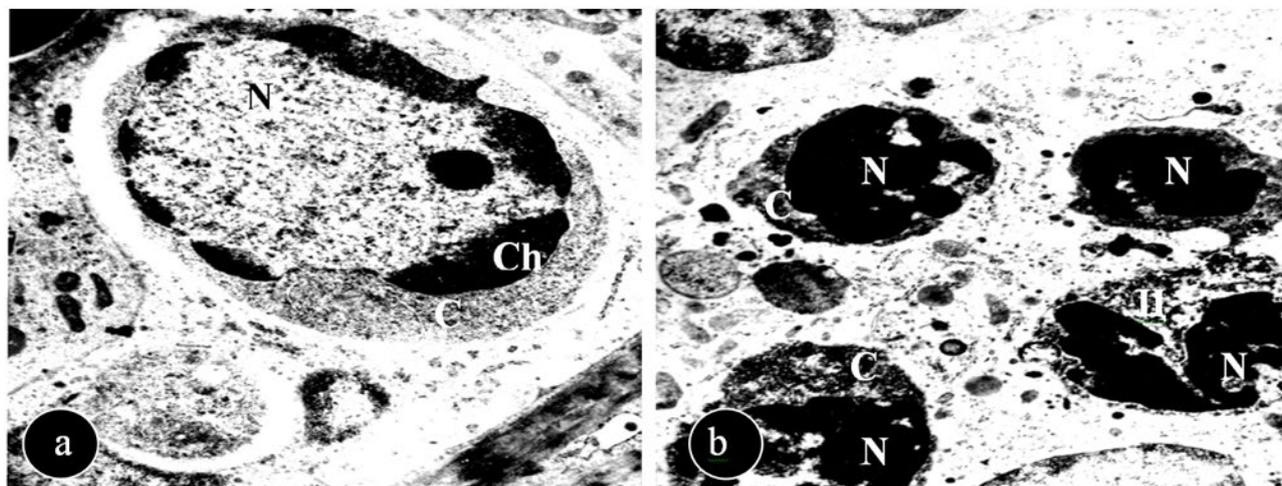


Fig. 2. Peyer's patches on the 7th day of the experiment after the immunodeficiency was corrected by imunofan (a - cell at the early stage of apoptosis, b - necrosis): N - nucleus, C - cytoplasm, Ch - chromatin. Magnification $\times 12,000$

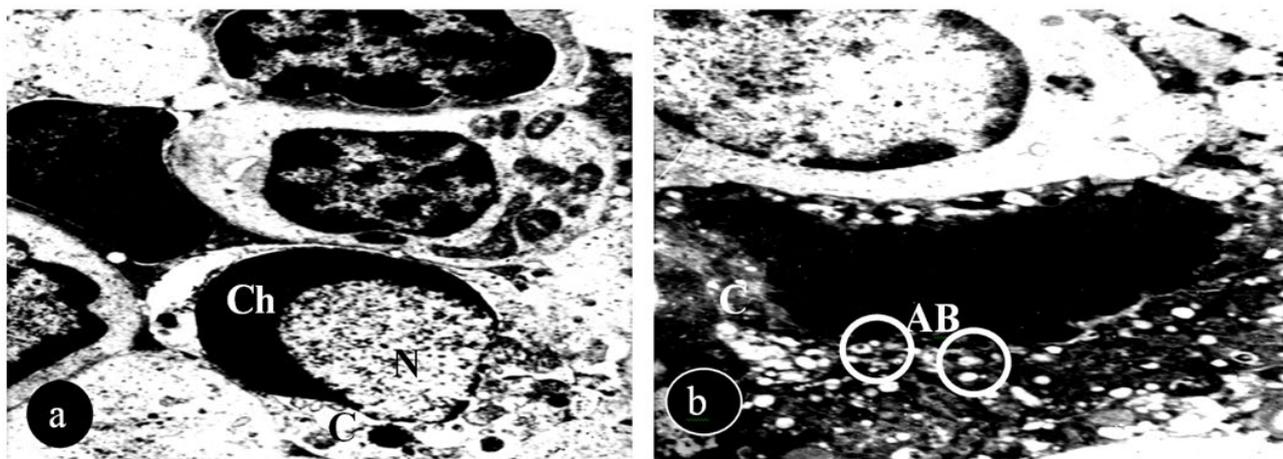


Fig. 3. Peyer's patches on the 30th day of the experiment after exposure of imunofan (a - early stage of apoptosis, b - late stage of apoptosis): N - nucleus, C - cytoplasm, Ch - chromatin, AB - apoptotic bodies. Magnification $\times 12,000$

On the 30th day of the experiment (**Fig. 3**) in animals of group I, the number of cells with apoptosis increases, while in group III, this process is detected only in isolated lymphocytes. In the early stages of apoptosis, cells are characterized by a decrease in their volume, condensation of chromatin under the nuclear membrane in the form of a demilune.

At the same time, the integrity of the membrane is preserved, the organelles look morphologically intact. At a later stage, the cells appear more electron-dense, their shrinkage continues. Deep cell membrane patches are formed, which leads to the separation of cell fragments, that is, the formation of apoptotic bodies surrounded by a membrane, consisting of cytoplasm and tightly spaced organelles, with or without nuclear fragments.

In animals of group II, on the 30th day of the experiment, the number of cells with apoptosis increases, but to a lesser extent than in animals of group I.

It should be noted that the processes of apoptosis and necrosis in Peyer's patches of rats' small intestine

predominantly affect the actively proliferating population of lymphoid cells.

CONCLUSIONS

1. Accumulations of the immune tissue of white mongrel male rats small intestine of the reproductive period actively respond to exogenous influences.

2. Mostly one type of cell death - apoptosis is detected in group I animals on the 7th and 30th day of the experiment in Peyer's patches. The number of cells with apoptosis is directly proportional to the observation period, which seems to indicate the ability of imunofan to influence this process, maintaining the constancy of Peyer's patches cellular composition in small intestine.

3. In animals of group II, on the 7th day of the experiment, both apoptosis and necrosis take place, and on the 30th day it is mainly apoptosis. Apparently, this is due to the immunosuppressive effect of cyclophosphamide on the lymphocytes of Peyer's patches of the small intestine, which manifests itself in the early period of the experiment after correction by the immunomodulator.

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