

UDC 616-076.4:591.441:615.277.3

*S.A. Kashchenko,**I.V. Bobrysheva*State establishment "Lugansk State
Medical University", Lugansk**FEATURES OF ULTRAMICROSCOPIC
STRUCTURE OF THE SPLEEN IN RATS
OF DIFFERENT PERIODS OF
POSTNATAL ONTOGENESIS UNDER
CONDITIONS OF EXPERIMENTAL
IMMUNOSUPPRESSION****Key words:** rats, spleen, white pulp,
cyclophosphamide.

Abstract. Aim of research is to study the features of ultramicroscopic structure of the white pulp of the spleen in the rats at the different stages of postnatal ontogenesis after introduction of immunosuppressive drug cyclophosphamide. 180 white noninbred male rats of three age periods: puberty (1st group), reproductive (2nd group) and period of the expressed senile changes (3rd group) served as a material for investigation. Cyclophosphamide was injected in a dosage resulting in immune depression (200 mg/kg of body weight of animal). Study of ultrastructural characteristics of white pulp of the spleen was performed on ultrathin sections with electron microscope. It was established that introduction of cyclophosphamide results to morphological alteration of spleen of white rats as a hypoplasia of the white pulp. The highest level of reactivity of the spleen in reply to introduction of cytostatic drug is observed at the animals of the 1st group. Changes of morphological characteristics of the white pulp of the spleen in the rats of the 2nd and the 3rd groups take place on a background of the evolutionary changes of organ. In the animals of the 2nd and the 3rd groups after the cyclophosphamide administration high activity of apoptotic processes in the white pulp of the spleen induced by cytostatic drug occurring against the backdrop of involutive changes in the organ is observed.

Introduction

The immune system is one of the most sensitive systems of the organism which quickly reacts to external influences. In humans and other mammals the spleen is the largest lymphoid organ, taking part in the development and maintenance of the cellular and humoral immune responses, the innate and adaptive immunity, quantitative and qualitative content of blood immune cells, lymph and other lymphoid organs [4, 5]. For the treatment of malignant tumors, autoimmune diseases, in transplantology there is the need of the immunosuppressive therapy which has a negative effect on various organs and tissues including the organs of the immune system [8].

Despite the presence of the numerous researches devoted to the structure of the spleen under the influence of external factors [1, 2, 3, 4, 7], questions of morphological changes in lymphoid tissue of the spleen under the action of immunomodulative drugs on the organism remain insufficiently studied.

The aim of research was to study the features of ultramicroscopic structure of the white pulp of the spleen in the rat of the different periods of postnatal

ontogenesis after administration of the immunosuppressive and cytostatic drug cyclophosphamide.

Materials and Methods

180 white nonbred male rats of three age periods: puberty (mass of 30-50 g) - the 1st group, reproductive (130-150 g) - the 2nd group and period of the expressed senile changes (300-330 g) - the 3rd group obtained from a vivarium of Lugansk State Medical University served as a material for investigation. Cyclophosphamide was singly administered to experimental animals intramuscularly in a dosage of 200 mg/kg of body weight. Control animals received 0,9% soluble sodium chloride. Animals were killed by decapitation under ethereal anaesthesia in 1, 7, 15, 30 and 60 days after drug introduction. The animals were cared for in accordance with the principles of the Guide to the Care and Use of Experimental Animals stated in the Helsinki declaration. For electron microscopy the spleen was removed and fixed immediately in 2,5% glutaraldehyde in 0,1 M phosphate buffer (pH 7,2). Specimens were postfixed in 2% aqueous osmium

tetraoxide, dehydrated through graded concentrations of ethanol and embedded in Araldite. Ultrathin sections were cut with ultramicrotome and mounted on copper grids. Sections were examined with electron microscope.

Results

High sensitivity of the white pulp cells of the spleen of the experimental animals to introduction of cyclophosphamide has been established.

So, studying of the spleen of the rats of the 1st group the data obtained have showed that in 1 and 7 days after administration of cyclophosphamide significant changes in all structural components of lymph nodules of white pulp occur.

In periarterial zone there are mainly small and medium lymphocytes having signs of destructive and dystrophic changes of the nucleus and cytoplasm. Cells contain irregular nuclei, rough endoplasmic reticulum (RER) of the cytoplasm contains extended cisterns, mitochondria have light matrix and partially damaged cristae.

In the mantle zone of the lymphatic nodule dark, irregular in shape lymphocytes with bulgings of plasmolemma, containing osmiophil nucleus with invaginations of karyolemma are observed. In the marginal zone the number of modified plasma cells with eccentrically located deformed nuclei increases (fig. 1). In the cytoplasm of these cells fragmentation and expansion of cisterns of RER, Golgi complex (GC), and damage of mitochondria are revealed. In 15, 30 and 60 days after the introduction of cyclophosphamide there is a delymphatization of the periarterial lymphoid sheaths. Small and medium lymphocytes with the changed nuclei and light cytoplasm devoid of the organelles are visualized. In some plasma cells the fragmentation of the RER cisterns, destructive changes of the GC and the mitochondria occurs. Dendritic cells contain small, irregular, electron dense nuclei and damaged organelles in their cytoplasm.

At the electron microscopic study of the spleen of animals of the 2nd and the 3rd groups in the structure of the white pulp similar changes, which are characterized by a considerable polymorphism, are observed. Structural zones of lymphatic nodules contain small and medium lymphocytes, some of which show signs of destructive changes: irregular nuclei, light, oedematous cytoplasm, almost devoid of organelles (fig. 2). However, there are single lymphoblasts in the process of mitotic division (fig. 3).

In periarterial lymphoid sheaths the number of small and medium lymphocytes, plasma cells reduces. The cytoplasm of light lymphocytes are often devoided of the organelles.

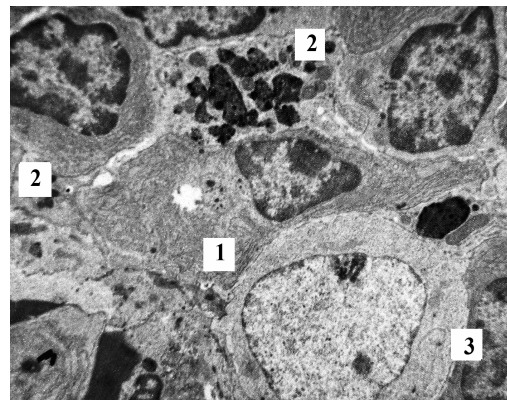


Fig. 1. Lymphatic nodule of the spleen of the rat of the 1st group in 7 days after injection of cyclophosphamide: 1 - plasma cell, 2 - macrophage, 3 - dendritic cell. Magnification x 12000

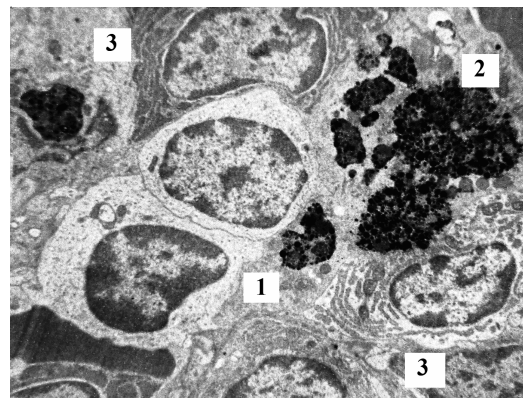


Fig. 2. Lymphatic nodule of the spleen of the rat of the 2nd group in 15 days after injection of cyclophosphamide: 1 - lymphocytes, 2 - macrophage, 3 - plasma cell. Magnification x 8000

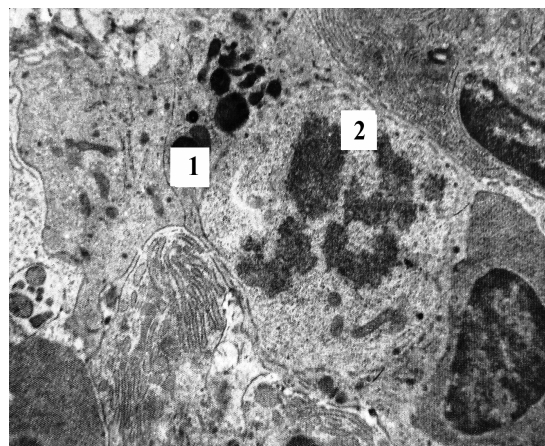


Fig. 3. Lymphatic nodule of the spleen of the rat of the 2nd group in 30 days after injection of cyclophosphamide: 1 - lymphoblast, 2 - mitosis. Magnification x 8000

The nuclei of individual plasma cells are irregular in shape, their cytoplasm contains fragments of RER, destructive changes of GC, in the mitochondria there is partial destruction of the cristae. Macrophages are small, with the increased electron density of the cytoplasm. In the cytoplasm of dendritic cells the damaged organelles are often

observed.

The typical morphological reflection of the influence of cyclophosphamide on the white pulp of the spleen of rats of these age periods is presence of lymphocytes with signs of apoptosis at periarterial and marginal zones of lymphatic nodules. Decrease in cell volume achieved by shrinking of the cytoplasm is observed. The cells contain dense nuclei, which have irregular contour, aggregation of chromatin as bodies of various shapes and sizes, knob-like protrusions, deep invaginations and constrictions of the karyolemma (fig. 4). In the later stages of apoptosis the fragmentation of the nucleus occurs.

Features of the ultramicroscopic structure of macrophages indicate their activation. Active macrophages have a number of finger-like projections of the cytoplasm, invaginations of plasmolemma, a large number of phagolysosomes and residual bodies in the cytoplasm. Size and electron density of

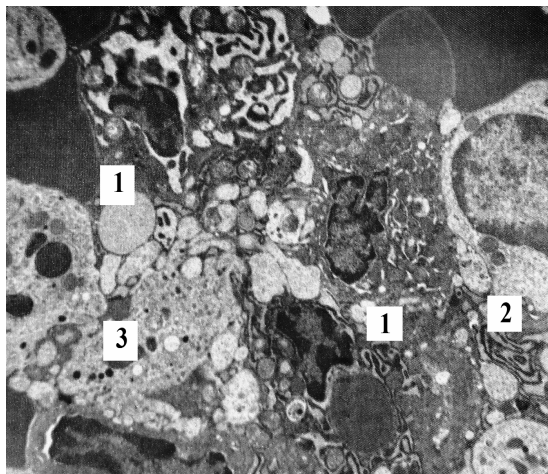


Fig. 4. Lymphatic nodule of the spleen of the rat of the 3rd group in 30 days after injection of cyclophosphamide: 1 - lymphocytes with signs of apoptosis, 2 - dendritic cell, 3 - macrophage. Magnification x 8000

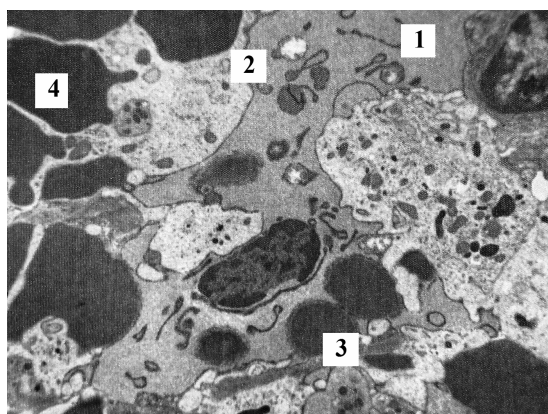


Fig. 5. Lymphatic nodule of the spleen of the rat of 3rd group in 30 days after injection of cyclophosphamide: 1 - macrophage, 2 - phagolysosomes, 3 - residues of apoptotic bodies, 4 - erythrocytes. Magnification x 8000

the phagocytosed material allow considering it as the residues of apoptotic bodies (fig. 5).

Macrophage activity may be related to their with the data of authors who noted increased apoptosis phenomena in the spleen of mice after exposure to low doses of gamma radiation [3]. Furthermore, according to the literature [9], spleen macrophages can trigger apoptosis by activation of enzymatic caspase cascade.

High activity of apoptosis in the white pulp of the spleen of rats of the 2nd and the 3rd experimental groups after cyclophosphamide injection may be the result of a combination of the activation of apoptosis by cytostatic drug and involutive processes of white pulp, the main feature of which in laboratory rats of the reproductive period, according to the literature [6], is increased apoptosis of lymphoid cells.

Conclusions

1. The rat spleen is characterized by a high degree of reactivity to the introduction of cyclophosphamide, which appears in hypoplasia of T- and B-zones of white pulp.

2. The lymphatic nodules are exposed to immunosuppressive influence of cyclophosphamide in the early periods after its injection, while periarterial lymphoid sheaths - in a long-term period.

3. Hypoplasia of the white pulp of the spleen of animals of the 1st group is associated with a reduction in the number of lymphocytes in lymphoid periarterial sheaths, and of the older age group - with increasing of apoptosis of lymphocytes in the lymphatic nodules.

4. The animals of the 1st group have a higher level of reactivity of white pulp of the spleen in response to the cytostatic drug than the rats of other age periods, which is manifested in the development of expressed destructive and dystrophic processes of lymphoid and stromal elements, which can be explained by certain morpho-functional immaturity of the spleen.

5. In the animals of the 2nd and 3rd groups after the administration of cyclophosphamide high activity of apoptotic processes in the white pulp of the spleen induced by cytostatic drug occurring against the backdrop of involutive changes in the organ is observed.

References. 1. Melechyn S.B., Chunareva M.B., Chetvertnykh V.A. *Morphologia - Morphology*, 2011, Vol. 140, no. 5, pp. 61-62 (in Russ.). 2. Moroz G.A., Kryventsov M.A. *Visnik problem biologii i meditsini - Bulletin of problems of biology & medicine*, 2011, Vol. 2, no. 2, pp.188-191 (in Russ.). 3. Motulyak A.P. *Klinichna anatomia ta operativna chirurgia - Clinical anatomy & operative surgery*, 2004, 2011, Vol. 3, no. 3, pp.29-31 (in Ukr.). 4. Nesterova A.A., Kapitonova M.Yu., Muraeva A.I., Krayushkin A.I., Chebnikov V.V., Chernov D.A., Smirnova T.S. *Morphologia - Morphology*, 2006, no. 5, pp.49-50 (in Russ.). 5. Prasolova L.A., Os'kina I.N.

Morphologia - Morphology, 2004, Vol. 125, no. 1, pp.59-63 (in Russ.). 6. Ryabikina A.I., Kapitonova M.Yu., Nesterova A.A., Morozova Z.Ch. Morphologia - Morphology, 2008, Vol. 132, no. 2, pp.58-61 (in Russ.). 7. Cherkasov E.V. Visnik morfologii - Bulletin of morphology, 2012, Vol. 18, no. 1, pp.6-10 (in Ukr.). 8. Colvin O. M. Alkylating agents. In Encyclopedia of Cancer 2 nd. Ed., Acad. Press, 2003, pp. 35 - 42. 9. Voll R.E., Yermann M., Roth E.A. Nature, 1997, V. 76, pp. 969 - 975.

ОСОБЛИВОСТІ УЛЬТРАМІКРОСКОПІЧНОЇ БУДОВИ СЕЛЕЗІНКИ ЩУРІВ РІЗНИХ ПЕРІОДІВ ПОСТНАТАЛЬНОГО ОНТОГЕНЕЗУ В УМОВАХ ЕКСПЕРИМЕНТАЛЬНОЇ ІМУНОСУПРЕСІЇ

С.А. Кащенко, І.В.Бобрішева

Резюме. Метою дослідження є вивчення особливостей ультрамікроскопічної будови білої пульпи селезінки щурів на різних етапах постнатального онтогенезу після введення імуносупресивної препарату циклофосфаміду. Матеріалом для дослідження слугували 180 білих беспородних щурів-самців трьох вікових періодів: статевого дозрівання (1-а група), репродуктивного (2-а група) і періоду виражених старечих змін (3-тя група). Циклофосфамід вводили дозою, яка призводить до імунної депресії (200 мг/кг ваги тіла тварини). Матеріал для електронномікроскопічного дослідження обробляли за загальноприйнятою методикою. Ультратонкі зрізи виготовляли на ультратонкотомі УМТП-4 Сумського ВО "Електрон", вивчали і фотографували під електронним мікроскопом ЕМ-125. Було встановлено, що введення циклофосфаміду призводить до морфологічних змін селезінки білих щурів у вигляді гіпоплазії білої пульпи. Найбільш високий рівень реактивності селезінки у відповідь на введення цитостатика спостерігається у тварин 1-ї групи. Зміна морфологічної характеристики білої пульпи селезінки у щурів 2 і 3 груп відбувається на тлі інволютивних змін органу. У тварин 2 і 3 груп після введення циклофосфаміду спостерігається висока активність апоптозних процесів у білій пульпі селезінки, індукованих введенням цитостатика, що відбувається на тлі інволютивних змін органу.

Ключові слова: щури, селезінка, біла пульпа, циклофосфамід.

ОСОБЕННОСТИ УЛЬТРАМИКРОСКОПИЧЕСКОГО СТРОЕНИЯ СЕЛЕЗЕНКИ КРЫС РАЗЛИЧНЫХ ПЕРИОДОВ ПОСТНАТАЛЬНОГО ОНТОГЕНЕЗА В УСЛОВИЯХ ЭКСПЕРИМЕНТАЛЬНОЙ ИММУНОСУПРЕССИИ

С. А. Кащенко, И. В. Бобрешева

Резюме. Целью исследования является изучение особенностей ультрамикроскопического строения белой пульпы селезенки крыс на разных этапах постнатального онтогенеза после введения иммуносупрессивного препарата циклофосфамида. Материалом для исследования послужили 180 белых беспородных крыс-самцов трех возрастных периодов: полового созревания (1-я группа), репродуктивного (2-я группа) и периода выраженных старческих изменений (3-я группа). Циклофосфамид вводили в дозе, приводящей к иммунной депрессии (200 мг/кг веса тела животного). Материал для электронномикроскопического исследования обрабатывали по общепринятой методике. Ультратонкие срезы изготавливали на ультратонкотоме УМТП - 4 Сумского ПО "Электрон", изучали и фотографировали под электронным микроскопом ЭМ-125. Было установлено, что введение циклофосфамида приводит к морфологическим изменениям селезенки белых крыс в виде гипоплазии белой пульпы. Наиболее высокий уровень реактивности селезенки в ответ на введение цитостатика наблюдается у животных 1-й группы. Изменение морфологической характеристики белой пульпы селезенки у крыс 2 и 3 групп происходит на фоне инволютивных изменений органа. У животных 2 и 3 групп после введения циклофосфамида наблюдается высокая активность апоптозных процессов в белой пульпе селезенки, индуцированных введением цитостатика, протекающих на фоне инволютивных изменений органа.

Ключевые слова: крысы, селезенка, белая пульпа, циклофосфамид.

State establishment "Lugansk State Medical University", Lugansk

Clin. and experim. pathol. - 2014. - Vol.13, №1 (47).-P.50-53.

Надійшла до редакції 01.02.2014

Рецензент – проф. Н.В.Пашковська

© S.A. Kashchenko, I.V. Bobrysheva, 2014