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CONDITION OF RAT' LIVER WITH EXPERIMENTAL IMMUNODEFICIENCY

Key words: *immunodeficiency, liver, hepatitis, enzymes.*

Abstract*. Aim of investigation is to determine the liver condition of rats with experimental immunodeficiency. Immune deficiency (ID) in rats was induced by the injection of the cytostatic cyclophosphamide. The presence of ID was determined by lymphocytes levels (in the blood) and lysocyme (in the serum and liver). The presence of hepatitis was identified by the levels of biochemical markers of liver inflammation (MDA, elastase) and cholestasis (ALP) as well as by the level of "liver markers" (ALT, bilirubin, alkaline phosphatase - ALP) in serum. Cyclophosphamide reduces the lymphocyte and lysocyme levels, and increases hepatic levels of inflammatory markers and cholestasis, and serum levels of "liver markers". Cyclophosphamide causes the development of immunodeficiency and hepatitis.*

Introduction

Immunodeficiency is an extremely common pathological condition arising as a result of various pathogenic factors (intoxication, radiation, viruses, nutritional disorders, genetic defects) and is characterized, above all, by a reduction in non-specific and specific antimicrobial protection [7, 1].

In the pathogenesis of pathological manifestations of immunodeficiency dysbiosis plays a crucial role, usually occurring as a result of weakening antimicrobial protection [4, 10].

The fundamental basis of dysbiosis is overgrowth of endogenous conditionally pathogenic, producing a number of microbial toxins, among which intestinal endotoxin lipopolysaccharide plays the most active role (LPS) [12, 13]. Joint action of different microbial toxins carries out not only the pathological effects on physiological systems of the body, but also induces activation of inflammatory and other protective systems of the body [12].

As formulated in recent years, the most important role in protecting against endogenous microbes is played by the liver, namely its antimicrobial function [8]. The level of the latter depends on the ability of the liver to be a reliable barrier to gut microbes and their toxins, and implement systemic inflammatory response to pathogenic factors.

The purpose of this study was to determine the condition of the liver in rats in which experimental immunodeficiency was induced by using the cytostatic cyclophosphamide, which is widely used in chemotherapy of malignant tumors.

Aim of investigation

To determine the liver condition of rats with experimental immunodeficiency.

Material and methods

Experiments were conducted on eighteen white Wistar rats (male, 10 months, live weight 280 ± 12 g), six of which were the control group, and 12 received two injections of the cytostatic cyclophosphamide 50 mg/kg solution intraperitoneally with interval of two days. Euthanasia of animals was carried out six at a time at 7 and 14 days under sodium thiopental anesthesia (20 mg/kg) by total exsanguination of the heart. We obtained serum and isolated the liver. In whole blood we tested for leukocytes and leukocyte formula [2]. Serum samples were tested for glucose [5], malondialdehyde (MDA) [3], elastase activity [3], catalase [3] and lysocyme [9] as well as the level of "liver markers" bilirubin [5] activity of alanine transaminase (ALT) [5] and the activity of alkaline phosphatase (ALP) [3]. In liver homogenate MDA, the activity of elastase, alkaline phosphatase, catalase and lysozyme were tested for. Using the ratio of catalase activity and MDA, we calculated antioxidant-prooxidant index - API [3]. The results were subjected to statistical analysis according to [6].

Results and Discussion

Table 1 shows the results of determination of the content of leukocytes, neutrophils and lymphocytes in the blood of animals injected with cyclophosphamide. As can be seen from these figures, seven days into the experiment the total number of white blood cells is reduced by almost threefold, while at 14 days is significantly increased, exceeding the rate of more than two times. The proportion of neutrophils in the composition of white blood cells in rats treated with cyclophosphamide was significantly increased after seven days, and the proportion of lymphocytes, ho-

Table 1

The content of neutrophils and lymphocytes in the blood of rats with immunodeficiency (M±m, n=6)

Parameter	Control	Immune deficiency Day 7	Immune deficiency Day 14
Leukocytes, g/L	14,0±2,3	4,5±1,2 p<0,01	29,0±6,4 p<0,05
Neutrophils, %	27,4±3,0	37,3±2,5 p<0,05	39,6±2,2 p<0,05
Lymphocytes, %	55,6±2,7	46,2±0,9 p<0,01	38,6±3,2 p<0,01
Lymphocytes/neutrophils	2,03±0,12	1,24±0,09 p<0,01	0,97±0,08 p<0,001

Note: In Tables 1-5: p – relative to the contro

wever, was significantly reduced in rats after administration of cyclophosphamide. The ratio of lymphocytes/neutrophils, which is a measure of immune deficiency [11] was significantly reduced in rats after administration of cyclophosphamide.

Table 2 presents data showing biochemical parameters of serum in rats which were administered cyclophosphamide. This data show that immune deficiency significantly increases the level of glucose, inflammatory markers, MDA and elastase, but significantly reduces the activity of protective enzymes: catalase and lysocyme. It can be said that immune deficiency causes the development of systemic inflammation [4, 10, 12].

Table 3 shows that administration of cyclophosphamide increases levels of inflammatory mar-

Table 2

Biochemical parameters of serum of rats with immunodeficiency (M±m, n=6)

Parameter	Control	Immune deficiency Day 7	Immune deficiency Day 14
Glucose, mmol/L	6,95±0,46	8,03±0,29 p<0,05	8,60±0,42 p<0,05
MDA, mmol/L	0,99±0,06	1,31±0,12 p<0,05	2,21±0,08 p<0,01
Elastase, mkat/L	0,26±0,01	0,32±0,02 p<0,05	0,34±0,01 p<0,05
Catalase, mkat/L	0,28±0,02	0,22±0,01 p<0,05	0,20±0,02 p<0,05
Lysocyme, U/L	85±2	51±5 p<0,001	45±5 p<0,001

Table 3

The level of markers of inflammation and cholestasis in rat liver with immunodeficiency (M±m, n=6)

№	Group	MDA, mmol/kg	Elastase, mkat/kg	ALP, mkat/kg
1	Control	110,0±12,0	0,45±0,01	7,10±0,78
2	Immune deficiency Day 7	167,8±11,1 p<0,05	0,58±0,04 p<0,05	9,56±0,96 p>0,05
3	Immune deficiency Day 14	228,9±9,5 p<0,01	0,66±0,02 p<0,01	9,30±0,96 p>0,05

kers in the liver (MDA and elastase) and cholestasis (ALP), which indicates the development of hepatitis. In favor of this evidence "liver markers" in serum are also indicative (Table 4). All three markers were significantly elevated in rats in which immunodeficiency was modeled.

We considered that the development of hepatitis in rats treated with cyclophosphamide, is the result of significantly (3, 5 - 6 times) reduction in nonspecific immunity, as evidenced by determining the activity of lysocyme (Table 5).

Thus, the investigation we conducted studying the liver when modeling experimental immunodeficiency give reasonable grounds for recommending the use of hepatoprotector drugs in patients with immunodeficiency, particularly during chemotherapy of

Table 4

The level of "liver" markers in the serum of rats with immunodeficiency (M±m, n=6)

№	Group	Bilirubin, μmol/L	ALT, μkat/L	ALP, μkat/L
1	Control	4,17±0,27	0,33±0,01	1,20±0,33
2	Immune deficiency Day 7	4,03±0,14 p>0,5	0,44±0,03 p<0,05	2,34±0,33 p<0,05
3	Immune deficiency Day 14	5,58±0,54 p<0,05	0,46±0,03 p<0,05	3,22±0,52 p<0,05

Table 5

Activity of lysocyme and catalase in rat liver with immunodeficiency (M±m, n=6)

№	Group	Lysocyme, U/kg	Catalase, mkat/kg
1	Control	83±20	6,27±0,10
2	Immune deficiency Day 7	25±12 p<0,05	6,16±0,06 p>0,3
3	Immune deficiency Day 14	14±6 p<0,01	6,18±0,08 p>0,3

malignant tumors.

Conclusions

1. Administering cyclophosphamide causes the development of immunodeficiency, as evidenced by a significant decrease in the number of lymphocytes and lysocyme activity.

2. The result of the immune deficiency is the development of hepatitis and systemic inflammation.

3. It can be considered necessary to prescribe hepatoprotectors in patients with immunodeficiency conditions.

References. 1. Алешина Р.М. Синдром вторичной иммунной недостаточности: клинико-лабораторная характеристика / Р.М. Алешина // Клін. імунологія. Алергологія. Інфектологія. - 2007. - №2 (07). - С. 17 - 20. 2. Базарнова М.А. (ред.). Руководство по клинической лабораторной диагностике. Ч. 1 / М.А. Базарнова. - К.: Вища школа, 1981. - С. 55.3. Биохимические маркеры воспаления тканей ротовой полости: методические рекомендации / А.П. Левицкий, О.В. Деньга, О.А. Макаренко [и др.]. - Одесса, 2010. - 16 с. 4.Воеводин Д.А. Дисбактериоз и иммунопатологический процесс / Д.А. Воеводин, Г.Н. Розанова, М.А. Стенина // ЖМЭИ. - 2005. - №2. - С.89 - 92. 5. Горячковский А.М. Клиническая биохимия / А.М. Горячковский. - Одесса: Экология, 2005. - 616 с. 6. Лапач С.Н. Статистические методы в медико-биологических исследованиях с использованием Excel / С.Н. Лапач, А.В. Чубенко, П.Н. Бабич. - К.: Морион, 2000. - 320 с. 7. Лебедев К.А. Иммунная недостаточность (выявление и лечение) / К.А. Лебедев, И.Д. Понякина. - М.: Медицина, Н.Новгород: НГМА, 2003. - 443 с. 8. Левицкий А.П. Антимикробная функция печени / А.П. Левицкий, С.А. Демяненко, Ю.В. Цисельский. - Одесса: КП ОГТ, 2011 - 141 с. 9. Левицкий А.П. Лизоцим вместо антибиотиков / А.П. Левицкий. - Одесса: КП ОГТ, 2005. - 74 с. 10. Роль иммунодефицита в развитии осложнений при вакцинации детей БЦЖ-вакциной / Л.И. Краснопрошина, Т.А. Севастьянова, В.А. Аксенова [и др.] // ЖМЭИ. - 2013. - № 6. - С. 50-55. 11. Чередеев А.Н. Современные подходы к диагностике иммунопатологических состояний / Лаб. дело. - 1998. - № 3. - С. 21-26. 12. Яковлев М.Ю. Элементы эндотоксиновой теории физиологии и патологии человека / М.Ю. Яковлев / / Физиол. человека. - 2003. - т. 29, №4. - С. 98-109. 13. Wang X. Endotoxins: structure, function and recognition / X. Wang, P. Quinn // Seria: Subcellular Biochemistry. - v. 53. - Springer, 2010. - 415 p.

СТАН ПЕЧІНКИ У ЩУРІВ З ЕКСПЕРИМЕНТАЛЬНИМ ІМУНОДЕФІЦИТОМ

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Резюме. Метою дослідження є вивчення стану печінки у щурів із експериментальним імунодефіцитом. Імунодефіцит у щурів був спровокований введенням цитостатика циклофосфану, який широко використовують при хіміотерапії злоякісних пухлин. Про розвиток імунодефіциту під дією циклофосфану свідчить зниження в крові рівня лім-

фоцитів та лізоциму. В печінці підвищується рівень маркерів запалення, а в сироватці крові - рівень "печінкових" маркерів, що вказує на розвиток гепатиту.

Ключові слова: імунодефіцит, печінка, гепатит, ферменти.

СОСТОЯНИЕ ПЕЧЕНИ У КРЫС С ЭКСПЕРИМЕНТАЛЬНЫМ ИММУНОДЕФИЦИТОМ

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Резюме. Целью исследования является изучение состояния печени у крыс с экспериментальным иммунодефицитом. Иммунодефицит у крыс был спровоцирован введением цитостатика циклофосфана, который широко используется в химиотерапии злокачественных новообразований. О развитии иммунодефицита под действием циклофосфана свидетельствует снижение в крови уровня лимфоцитов и лизоцима. В печени повышается уровень маркеров воспаления, а в сыворотке крови - уровень "печеночных" маркеров, что указывает на развитие гепатита.

Ключевые слова: иммунодефицит, печень, гепатит, ферменты.

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