Реферати

ТЕРМОЦИКЛЮВАННЯ ЯК МЕТОД ДЕЗІНТЕГРАЦІЇ BIFIDOBACTERIUM BIFIDUM

Книш О.В., Пахомов О.В., Погоріла М.С., Савінова О.М., Балак О.К.

У дослідженні порівнюється пошкоджуючий вплив двох методів термоциклювання на пробіотичний штам *B. bifidum*. Суспензії свіжовиділених біфідобактерій і бактерій, що зберігалися за гіпотермічних умов протягом 24 годин, піддавали десятикратному термоциклюванню двома способами, які передбачали повільне охолодження зразків до (-23 ± 1) °С або швидке охолодженням до (-196 ± 1) °С з подальшим відігріванням на водяній бані при 37 °С до повного відтавання. Виживання клітин оцінювали шляхом підрахунку колонієутворюючих одиниць і проточної цитометрії з використанням карбоксифлуоресцеїну діацетату (кФД) як флуорохрому. Термоциклювання з повільним охолодженням до (-23 ± 1) °С виявило більш виражену дезінтегруючу дію на біфідобактерії. Попереднє зберігання біфідобактерій за гіпотермічних умов не значно підвищувало їх стійкість до дезінтегруючої дії термоциклювання.

Ключові слова: біфідобактерії, термоциклювання, виживаність, проточна цитометрія, дезінтеграція.

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ТЕРМОЦИКЛИРОВАНИЕ КАК МЕТОД ДЕЗИНТЕГРАЦИИ BIFIDOBACTERIUM BIFIDUM

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В исследовании сравнивается повреждающее действие двух методов термоциклирования на пробиотический штамм *B. bifidum*. Суспензии свежевыделенных бифидобактерий и бактерий, хранившихся в гипотермических условиях в течение 24 часов, подвергали десятикратному термоциклированию двумя способами, которые предполагали медленное охлаждение образцов до (-23 ± 1) °С или быстрое охлаждением до (-196 ± 1) °С с последующим отогревом на водяной бане при 37 °С до полного оттаивания. Выживаемость клеток оценивали путем подсчета колониеобразующих единиц и проточной цитометрии с использованием карбоксифлуоресцеина диацетата (кФД) в качестве флуорохрома. Термоциклирование с медленным охлаждением до (-23 ± 1) °С оказывало более выраженное дезинтегрирующее действие на бифидобактерии. Предварительное хранение бифидобактерий в гипотермических условиях не значительно повышало их устойчивость к дезинтегрирующему действию термоциклирования.

Ключевые слова: бифидобактерии, термоциклирование, выживаемость, проточная цитометрия, дезинтеграция.

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MORPHOLOGICAL FEATURES OF DOXORUBICIN-INDUCED LIVER DAMAGE ASSOCIATED WITH NONALCOHOLIC STEATOHEPATITIS

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The paper consideres the study of histological features of anthracycline-induced liver lesions concomitant with non-alcoholic steatohepatitis.The findings of the study have established the presence of moderate fatty degeneration of the liver with mild focal protein dystrophy of hepatocytes in the lobules of the animals with experimental non-alcoholic steatohepatitis. In the group of animals with anthracycline-induced liver damage, moderate periportal necrosis of hepatocytes along with a mild small-droplet fatty degeneration. Prominent total (centrilobular and periportal) subacute liver necrosis along with moderate fatty degeneration was found in animals with anthracycline-induced liver damage associated with experimental non-alcoholic steatohepatitis.

Keywords: non-alcoholic steatohepatitis, anthracycline-induced liver damage, rats.

The work is a fragment of the research project "Development of methods for prevention and treatment of the drug-induced damages of the internal organs", state registration No. 0115U001087.

Doxorubicin belongs to anthracycline antibiotics that are considered as one of the most effective antitumor drugs widely used in oncological and oncohematological clinical practice [5, 6, 9-11]. Doxorubicin is the most important mainstay in the treatment of breast cancer, soft tissue sarcoma and aggressive lymphomas of high malignancy, acute lymphoblastic and myeloblastic leukemias [6, 9-11]. In some cases, high toxicity of doxorubicin may be restriction on its use [5, 6, 8-11]. Moreover, the toxic effect of doxorubicin on the tissues of the heart, kidneys, liver has been confirmed [5, 9, 12-14]. Notably, damaging effect of anthracycline antibiotics is due to its specific pharmacokinetics. Doxorubicin is able to accumulate intracellularly in concentrations 10-500 times higher than extracellularly [8]. Another important point in the development of toxic effects of all cytostatics, including anthracyclines, is the impact on all cells, both malignant and healthy [7, 8]. Doxorubicin is metabolized mainly in the liver with the formation of a highly toxic metabolite of doxorubicinol, which has a direct damaging effect on liver tissue [6]. Histological manifestations of liver lesions induced by anthracycline antibiotics are characterized by necrosis and degeneration of hepatocytes, sinus dilatation, vascular stagnation and hemorrhage [6, 11, 13, 14].

Over the last decades experimental and clinical studies have been conducted to study the mechanisms of the organotoxic effect of doxorubicin. Thus, D. Chaudhary et al. [6] reported about the study of doxorubicin hepatotoxicity performed on 60 albino mature rats administered with doxorubicin at a single dose of 10 mg/kg body weight. Anatomical and histomorphological parameters were studied on day 7 and 14. Liver weight in rats treated with doxorubicin on day 14 of the study was significantly lower than in the control group. The diameter of hepatocytes, the size of the nucleus of the hepatocyte was greater both on day 7 and 14 compared to the control group ($p < 0.001$). Thus, the authors proved that the toxic effect of doxorubicin is delayed and increases with time. Similar changes in the histological structures of the liver were observed by a number of researchers who confirmed that doxorubicin causes the enlargement of hepatocytes and their nuclei, as well as the change in the shape of the hepatocyte nucleus and disruption of their membrane structure [6, 11, 13, 14].

We believe that the study of histomorphological features of liver lesions in the presence of concomitant nonalcoholic steatohepatitis (NASH), which may contribute to drug metabolism and potentiate the vulnerability of hepatocytes to the toxic effects of cytostatics is crucial [3, 4].

The purpose of the work was to study histological features of anthracycline-induced liver lesions concomitant with NASH.

Materials and methods. The study involved 30 mature outbred albino rats (male rats=15 (50%); female rats=15 (50%) weighted 160-220 g. The study was carried out in two stages.

At the first stage 10 rats (5 males and 5 females) were exposed to modeled NASH during 9 weeks (from day 1 to day 63), induced by a diet, containing 42,8% fats. The daily ration per one animal included combination fodder-concentrate granulated 0.04 kg, 72.5% butter 0.01 kg, refined sunflower oil 0.01 kg, palm oil 0.01 kg. Vegetables were excluded from the ration of the experimental animals. 4% aqueous solution of fructose was used as the sole source of liquid. Another 20 rats received a regular rations of vivarium, containing combination fodder-concentrate granulated 0.04 kg, low-fat cheese 0.006 kg, carrots 0.02 kg, cabbage 0.015 kg per one animal a day for 9 weeks (from day 1 to day 63).

At the second stage of the experiment the modeling of doxorubicin- induced liver damage was carried out. Experimental rats were divided into 3 groups:

Rats of Group I (n=10; males n=5; females n=5) were exposed to modeled NASH, induced by a high-calorie diet from day 1 to day 63, followed by administration of 5mg/kg/day doxorubicin intraperitoneally for 3 days (from day 64 to day 66) to reach the cumulative doze of 15 mg/kg;

Rats of Group II (n=10; males n=5; females n=5) were on a regular rations of vivarium from day 1 to day 63, followed by administration of 5mg/kg/day doxorubicin intraperitoneally from day 64 to day 66 to reach the cumulative doze of 15 mg/kg;

Rats of Group III (n=10; males n=5; females n=5) were on a regular rations of vivarium from day 1 to day 63, followed by administration of intraperitoneal 0.9% sodium chloride solution at a dose of 1 ml for 3 days.

Euthanasia of rats was performed under 50 mg/kg thiopental anesthesia on day 67 of the experiment. The obtained fragments of the liver were fixed in buffered 10 % formalin for 24 hours that were subsequently embedded into paraffin according to conventional technique [1]; sections of 5 µm thick were made and stained with hematoxylin and eosin.

Animal housing and experiments on them have been carried out in compliance with the principles of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” (Strasbourg,1986), “General Ethic Rules for Conducting Experiments on Animals”, adopted by the I National Congress on Bioethics [2] and the requirements of the “Procedure for conducting tests, experiments on animals by research institutions” (2012).

The study and documentation of the sections was carried out using the Biorex–3 BM–500T microscope in ×400 magnification, equipped with the DCM–900 digital microphoto attachment and software adapted for the above studies.

Results of the study and their discussion. The analysis of the histological sections of the liver of rats of the control group has found that it had a lobular structure. Central veins were detected in the center of each lobule. Hepatic beams formed by hepatocytes and sinusoidal capillaries between them were visualized radially. In the central zones of the lobules, binuclear hepatocytes were determined on the average 8.36 ± 0.08 in the field of view (fig. 1a).

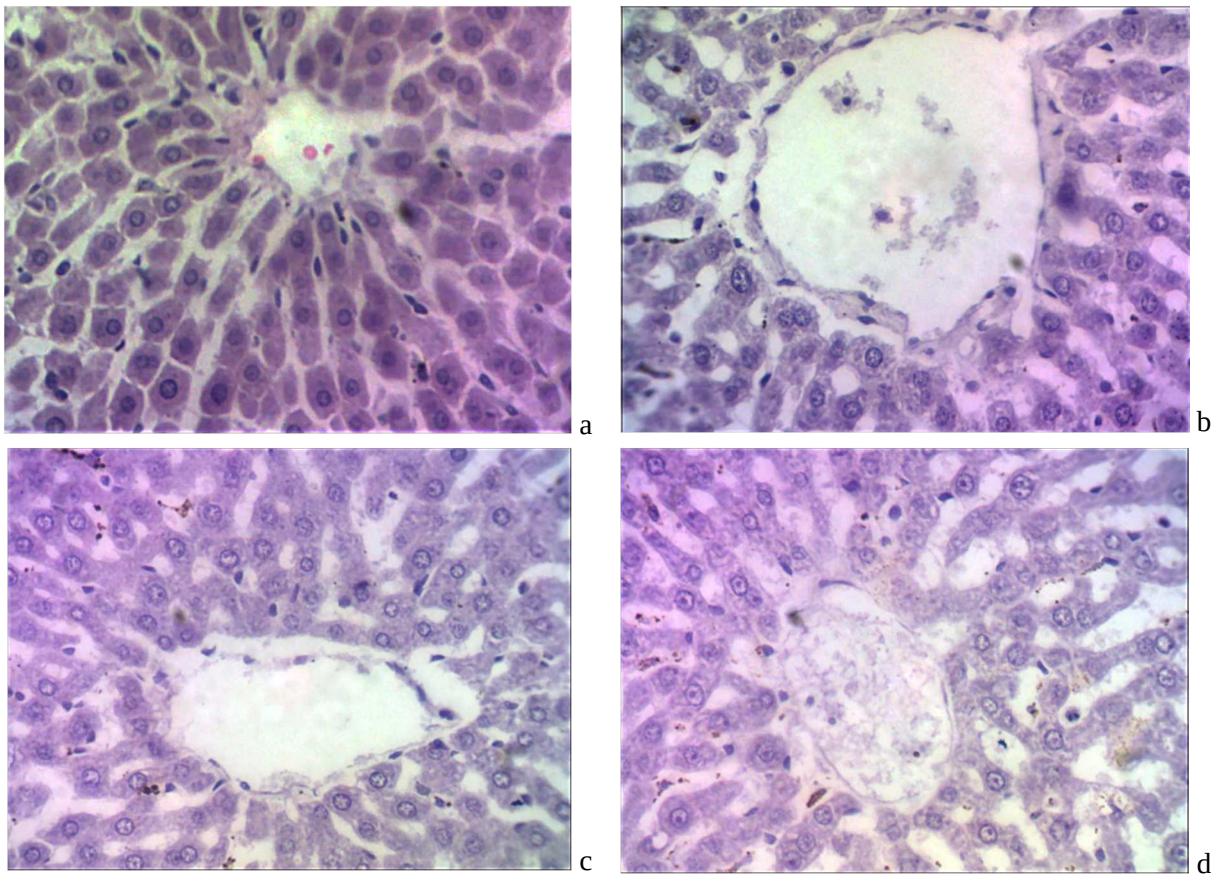

Fig. 1. Central part of the liver lobule of rat of control group (a), with experimental non-alcoholic steatohepatitis (b), with anthracycline-induced liver damage (c) and with anthracycline-induced liver damage associated with experimental non-alcoholic steatohepatitis (d). H&E stain. Lens:×40 magnification; Ocular lens:×10 magnification.

In rats with simulated experimental nonalcoholic steatohepatitis, varicose central veins were detected in the central areas of the lobules. The endothelium was thinned; the nuclei of endothelial cells were visualized in the form of thin basophilic stripes. Cellular detritus and sporadic squamous cells were noted in the lumens. Signs of amorphous hyperhydration were detected perivascularly. Sinusoidal capillaries were dilated with heterogeneous blood supply. Hepatocytes showed polymorphism and moderate manifestations of fatty degeneration. Binuclear cells were not visualized. Hepatocytes with pyknotically altered nuclei were detected locally (fig. 1b).

The study of histological sections of the liver of rats with anthracycline-induced liver damage has found that the central veins were dilated with morphological signs of edema, detected perivascularly. Sinusoidal capillaries were unevenly dilated and ischemic (fig. 1c).

The beam-radial structure of the lobules was preserved, but cells with pyknotically altered nuclei and karyorhexis phenomena, as well as with small-droplet fatty degeneration, were detected. The number of binuclear hepatocytes decreased compared to the control group of animals, accounting for 3.04 ± 0.02 in the field of view.

In the central parts of the lobules of the liver of rats with anthracycline-induced liver damage associated with experimental non-alcoholic steatohepatitis the central veins were dilated, the endothelium was thinned. The lumens were filled with inhomogeneous content of medium optical density. The sinusoidal capillaries were significantly dilated. The nuclei of the vast majority of hepatocytes were visualized in the state of karyopyknosis, karyorhexis and karyolysis. The cytoplasm showed cytolysis along with moderate small-droplet fatty degeneration (fig. 1d).

In the intermediate parts of the lobules of control rats, the shape of the hepatocytes was more orbicular and larger than the cells in the central parts of the lobules. Binuclear cells were sporadic. Uncondensed chromatin predominated in the nuclei and single centric nucleolus was determined (Fig. 2a).

In rats exposed to simulated experimental nonalcoholic steatohepatitis, dilated sinusoidal capillaries with inhomogenous blood supply were detected in the intermediate zones of the lobules. Hepatocytes were polymorphic with hydropic and fatty degeneration. Binuclear cells were not visualized. Hepatocytes with the phenomena of karyopyknosis were detected (fig. 2b).

Histological sections of the intermediate parts of the liver lobules of rats with anthracycline-induced liver damage showed that the sinusoidal capillaries were inhomogenously dilated, locally narrowed, and ischemic.

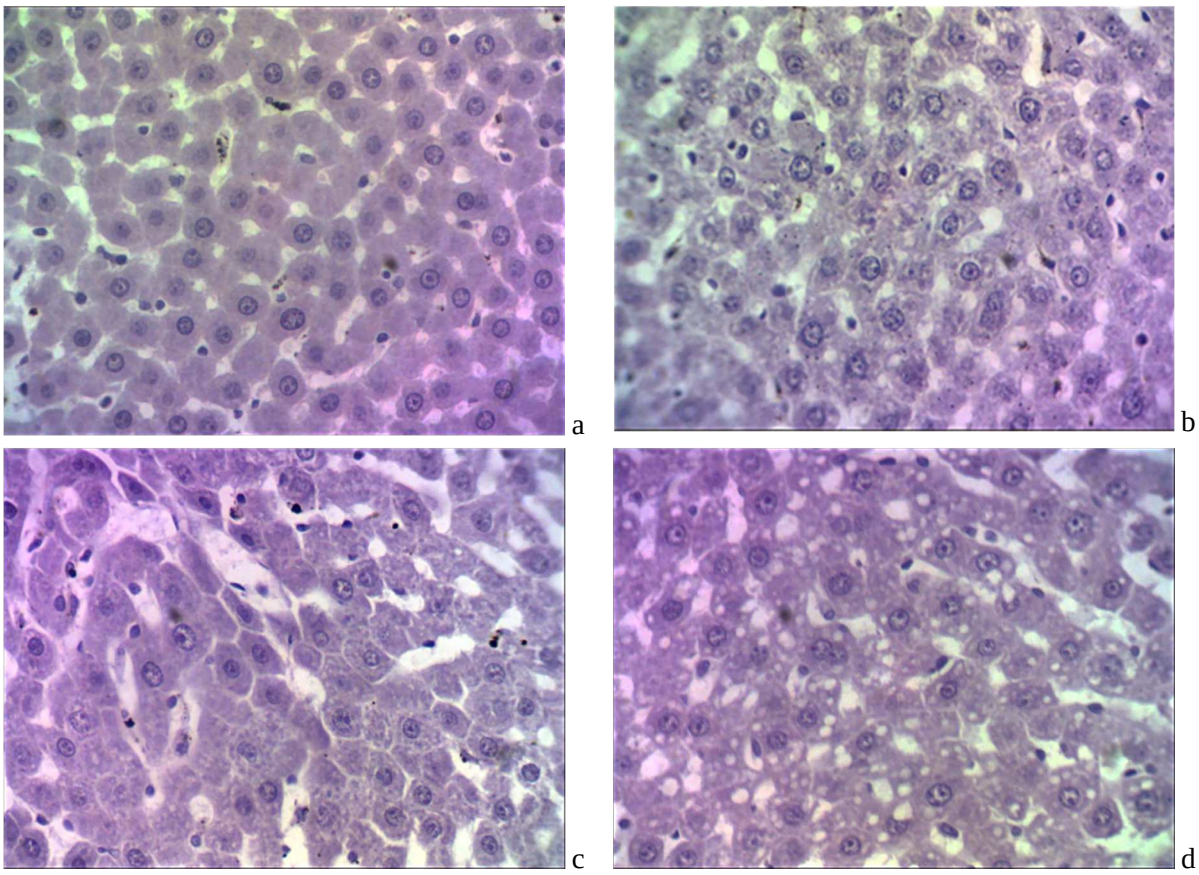

Fig. 2. Intermediate part of the lobule of the liver of rat of control group (a), with experimental non-alcoholic steatohepatitis (b), with anthracycline-induced liver damage (c) and with anthracycline-induced liver damage associated with experimental non-alcoholic steatohepatitis (d). H&E stain. Lens:×40 magnification; Ocular lens:×10 magnification.

The beam-radial structure of the lobules was preserved, but cells with a dark unstructured cytoplasm and compacted nuclei were detected. The vast majority of hepatocytes were with small-droplet fatty degeneration. Binuclear hepatocytes were not detected (fig. 2c).

In the intermediate parts of the lobules of the liver of rats with anthracycline-induced liver damage associated with experimental nonalcoholic steatohepatitis, sinusoidal capillaries were inhomogenously dilated; some of them were narrowed due to edema of hepatocytes, in the cytoplasm of which large vacuoles were found (fig. 2d).

Portal triads composed of the artery, vein and bile duct were localized on the periphery of the lobules in rats of the control group. The blood supply of the vascular system of the lobules was moderate. The number of binuclear hepatocytes was 2 ± 0.01 in the field of view. The vast majority of cells had a cubic shape (fig. 3a).

The study of histological sections of the liver of rats with experimental non-alcoholic steatohepatitis has found that in the peripheral parts of the liver lobules arterioles were spasmodic in the triads, nuclei of the endothelial cells protruded into the lumens. The surrounding connective tissue had morphological signs of hyperhydration of the amorphous substance and was infiltrated by leukocytes, namely, lymphocytes, macrophages and single segmentated leukocytes. The sinusoidal capillaries were inhomogenously dilated and ischemic. Hepatocytes were shrunken, the nuclei of the latter were pyknotic; small-droplet fatty degeneration was noted in the cytoplasm (fig. 3b).

In the experimental animals with anthracycline-induced liver damage, hepatocytes with dark cytoplasm and elongated cells dominated in the peripheral parts of the lobules. Karyopyknosis and karyolysis were observed in some cells. In the triads arteries were spasmodic and poorly expressed periportal edema was detected. Leukocyte infiltrates, where macrophages and lymphocytes prevailed, were visualized periportally (fig. 3c).

The study of histological sections of the liver of rats with anthracycline-induced liver damage associated with experimental non-alcoholic steatohepatitis has shown edema of the periportal stroma and its infiltration by macrophages and plasma cells were detected on the periphery of the lobules. Karyorhexis and karyopyknosis were noted in the vast majority of hepatocytes, as well as cytolysis in the cytoplasm. The preserved cells were visualized in a state of small-droplet hydropic degeneration (fig. 3d).

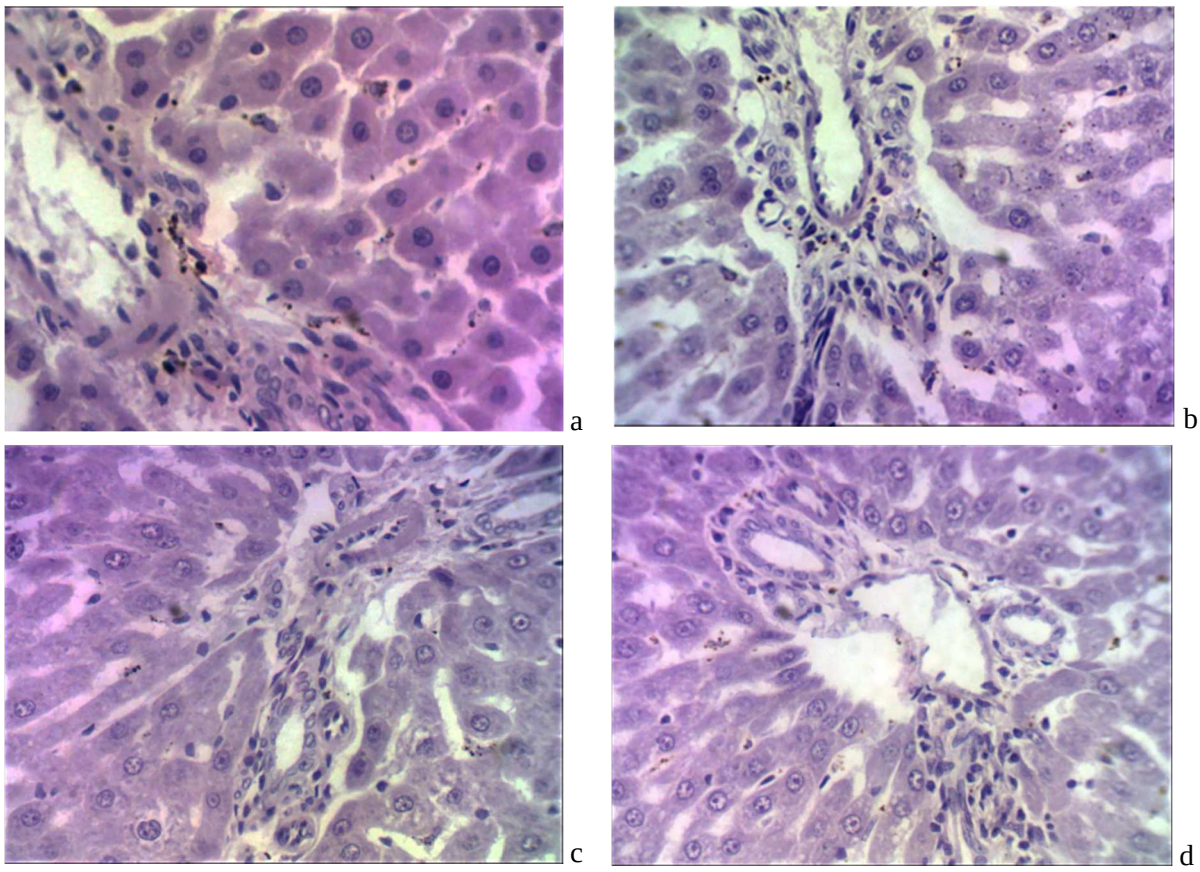

Fig. 3. Portal triad and peripheral portion of the liver lobule of the rat of control group (a), with experimental nonalcoholic steatohepatitis (b), with anthracycline-induced liver damage (c) and with anthracycline-induced liver damage associated with experimental nonalcoholic steatohepatitis (d). H&E stain. Lens:×40 magnification; Ocular lens:×10 magnification.

The findings of the study related to histofunctional changes in the liver in anthracycline-induced liver damage are consistent with the findings of other researchers [6, 11, 13, 14]. Non-alcoholic steatohepatitis contributes to the drug metabolism disorder, as well as potentiates the vulnerability of hepatocytes to the toxic effects of cytostatics [3, 4].

Conclusion

The findings of the study have established the presence of moderate fatty degeneration of the liver with mild focal protein dystrophy of hepatocytes in the lobules of the animals with experimental non-alcoholic steatohepatitis. In the group of animals with anthracycline-induced liver damage, moderate periportal necrosis of hepatocytes along with a mild small-droplet fatty degeneration. Prominent total (centrilobular and periportal) subacute liver necrosis along with moderate fatty degeneration was found in animals with anthracycline-induced liver damage associated with experimental non-alcoholic steatohepatitis.

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Реферати

МОРФОЛОГІЧНІ ОСОБЛИВОСТІ ДОКСОРУБІЦИН-ІНДУКОВАНИХ УРАЖЕНЬ ПЕЧІНКИ НА ТЛІ НЕАЛКОГОЛЬНОГО СТЕАТОГЕПАТИТУ

Маслова Г.С., Скрипник І.М., Єрошенко Г.А.

В роботі досліджено гістологічні особливості антрациклін-індукованих уражень печінки на тлі неалкогольного стеатогепатиту. В результаті проведеного дослідження встановлено, що у тварин з експериментальним неалкогольним стеатогепатитом у часточках печінки визначено помірно виражену жирову дистрофію печінки зі слабо вираженою осередковою білковою дистрофією гепатоцитів. В групі тварин з антрациклін-індукованим ураженням печінки встановлений помірний перипортальний некроз гепатоцитів на тлі слабко вираженої дрібнокрапельної жирової дистрофії. Виражений тотальний (центролобулярний і перипортальний) підгострий некроз печінки на тлі помірно вираженої жирової дистрофії виявлений у тварин з антрациклін-індукованим ураженням печінки на тлі експериментального неалкогольного стеатогепатиту.

Ключові слова: неалкогольний стеатогепатит, антрациклін-індуковані ураження печінки, щурі.

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МОРФОЛОГИЧЕСКИЕ ОСОБЕННОСТИ ДОКСОРУБИЦИН-ИНДУЦИРОВАННОГО ПОРАЖЕНИЯ ПЕЧЕНИ НА ФОНЕ НЕАЛКОГОЛЬНОГО СТЕАТОГЕПАТИТА

Маслова Г.С., Скрипник И.М., Ерошенко А.

В работе исследованы гистологические особенности антрациклин-индуцированных поражений печени на фоне неалкогольного стеатогепатита. В результате проведенного исследования установлено, что у животных с экспериментальным неалкогольным стеатогепатитом в дольках печени выявлено умеренно выраженную жировую дистрофию печени со слабо выраженной очаговой белковой дистрофией гепатоцитов. В группе животных с антрациклин-индуцированным поражением печени установлен умеренный перипортальный некроз гепатоцитов на фоне слабо выраженной мелкокапельной жировой дистрофии. Выраженный тотальный (центролобулярный и перипортальный) подострый некроз печени на фоне умеренно выраженной жировой дистрофии обнаружен у животных с антрациклин-индуцированным поражением печени на фоне экспериментального неалкогольного стеатогепатита.

Ключевые слова: неалкогольный стеатогепатит, антрациклин-индуцированные поражения печени, крысы.

Рецензент Старченко І.І.

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REACTION OF HEMOMICROCIRCULATORY BED OF RAT LIVER AND CHANGES IN THE FUNCTIONAL STATE OF THE NITRIC OXIDE CYCLE UNDER THE CONDITIONS OF MODELING ALCOHOLIC HEPATITIS

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The purpose of the work was to study the changes in the hemomicrocirculatory bed of the liver and the role of the NO-ergic system in their development under the conditions of modeling alcoholic hepatitis. At the early stages of modeling alcoholic hepatitis, the thickness of the vascular wall of the central vein, interlobular artery and lobular arterioles increases, while the thickness of the vascular wall of the interlobular vein, the lobular venule and the sublobular vein decreases. These changes are associated with dysregulatory changes in the nitric oxide cycle in rat liver. Dysregulatory changes are manifested by an increase in the activity of inducible and constitutive isoforms of NO synthases against the background of decreased activity of arginases in the absence of statistically significant changes in the activity of nitrate and nitrite reductases in the liver of rats with simulated alcoholic hepatitis.

Key words: liver, alcoholic hepatitis, nitric oxide cycle, rats.

The work is a fragment of the research project "Peculiarities of pathological changes development in digestive system organs and development of their correction methods", state registration No. 0120U100502.

Alcohol consumption is the seventh leading risk factor for various diseases, injuries and death. In 6.8% of deaths among men and 2.2% among women, the cause is alcohol abuse. The total cost of eliminating the social consequences of alcohol consumption makes more than 1% of the gross national product for high- and middle-income countries, much higher than the budget for health care [5].