Volodymyr Nadykto *Editor*

Modern Development Paths of Agricultural Production

Trends and Innovations

Springer

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ISBN 978-3-030-14917-8 ISBN 978-3-030-14918-5 (eBook)
https://doi.org/10.1007/978-3-030-14918-5

Library of Congress Control Number: 2019935551

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

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Modification of Modeling Method of Toxic Dystrophy of Liver in Rats

Viktoriya Gryshchenko, Olena Danchenko and Viktoriya Musiychuk

1 Introduction

The liver is the largest digestive gland that plays an important role in providing homeostasis of the internal environment of the body and in the development of adaptive reactions, which is due to its participation in many metabolic processes, anatomical, and functional connections with other organs and systems of the body. Recently, the risk of developing hepatopathy is increasingly associated with irrational pharmacotherapy of the underlying disease [4, 23, 24]. Thus, the administration of tetracycline antibiotics (doxycycline, chlortetracycline, metacycline) causes the occurrence of mitochondrial cytopathies. Toxic hepatitis can occur both on the first day of taking an antibacterial drug and after a few months from the start of treatment. To determine the degree of damage to the liver parenchyma, it is recommended to study the activity of enzymes with various intracellular topographies [7, 11, 16, 19].

Clinical and morphological manifestations of toxic liver lesions are diverse [20]. There are three main types of damage: hepatocellular, cholestatic, and mixed. The target of toxic effects can be hepatocytes (dystrophy, necrosis), bile ducts and tubules (cholestasis), or sinusoidal cells (endothelium). Medication of the liver, in particular parenchyma damage in the form of functional disorders (induction of microsomal enzymes, hyperbilirubinemia), lead to necrosis or apoptosis. Other hepatotoxic effects of pharmacotherapy include the formation of steatosis in the form of acute fat changes, steatohepatitis, cholestasis, granulomatous changes and damage to the vascular system of the liver, etc. [3, 5, 17].

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V. Nadykto (ed.), *Modern Development Paths of Agricultural Production*,
https://doi.org/10.1007/978-3-030-14918-5_67

A significant compensatory antitoxic reserve of the body neutralizes the negative impact of toxic substances [9, 18]. In the liver, which performs a detoxification function, their inactivation and binding take place for further elimination. The realization of this function during the first phase depends significantly on the activity of cytochrome P450. With the participation of the corresponding enzymatic systems, oxidation, reduction, hydrolysis, hydration, and dehalogenation of toxins occur [10]. In the next phase is registered conjugation of toxic substances and their deactivation due to the processes glucuronidation, acetylation, methylation, binding with amino acids and glutathione. However, the detoxification capacity of the organ is significantly reduced if the influence of harmful factors develops against the background of hepatopathology. The severity and severity of symptoms are determined by the dose of xenobiotics [2].

Morphological heterogeneity of toxic lesions of the liver and preferential localization of necrosis causes metabolic zoning. Behind metabolic activity, hepatocytes are heterogeneous. Their zoning for a difference in metabolic functions determines the selective sensitivity of hepatocytes to various pathological factors. In particular, cells of zone I contain more mitochondria, oxidative processes, gluconeogenesis, synthesis of cholesterol, urea, and bile acids are more intensive. In hepatocytes of the III zone, glycolysis, lithogenesis, cytochrome P450-dependent hydroxylation, and glucuronidation of xenobiotics are most pronounced. The use of tetracycline antibiotics leads to the development of necrosis of hepatocytes predominantly of the III zone [4].

Toxic liver damage due to taking medications presents a certain diagnostic complexity [1, 15]. Sometimes, with prolonged therapy, they proceed without clinical manifestations, which indicate chronic intoxication. This leads to a comprehensive study of the pathogenesis of the development of drug hepatopathology, primarily at the molecular level, the creation of sensitive test systems for early diagnosis, as well as the means of prevention and therapy of such conditions [6]. Therefore, it remains relevant to develop methods for the artificial reproduction of pathological conditions of the liver due to its medicinal damage. We have already developed a method for modeling drug-induced hepatopathy by introducing into the body of rats a non-steroidal anti-inflammatory drug diclofenac sodium, which provokes the development of toxic hepatitis [12].

The purpose of this work was the artificial reproduction in laboratory rats of an acute form of toxic hepatodystrophy without sudden changes in the clinical state, which corresponds to the spontaneous course of this hepatopathology in animals with respect to the complex of general clinical, biochemical, morphological, and pathoanatomical changes, but does not lead to death.

2 Experimental Studies

2.1 Research Method and Materials

The Modeling of Toxic Hepatodystrophy. The experiment involved white laboratory rats (males) who were selected according to the principle of analogues with a body weight of 200–220 g. With the known scheme for modeling fatty hepatosis in laboratory rats by oral administration of 1% solution of tetracycline hydrochloride at a dose of 500 mg/kg, for 5 days, an extremely severe clinical condition is observed, which develops already on the 2–3th day of the experiment, a lethal outcome is possible [13]. In addition, this type of animal with a body weight of 200–220 g is difficult to perceive the recommended volume of tetracycline hydrochloride solution, which borders or even exceeds the physiological capabilities of the stomach, and this in most cases is 5 cm^3 or more. This was the basis for work on improving the existing method for modeling the acute form of toxic hepatodystrophy. For this, two groups (control and study) were formed in twenty rats each. The animals of the study group artificially reproduced the acute form of hepatodystrophy by the method [9] of intragastric injection of the 4% solution of tetracycline hydrochloride, modified by us, using a probe at a dose of 250 mg/kg once a day, for 7 days in accordance with the current standardization and quality criteria conducting biological experiments and biomodeling principles [21]. Animals of the study group remained during the experiment without treatment. In the control group, clinically healthy rats were treated, with an equivalent volume of distilled water administered intragastrically by means of a probe.

The rats were separately placed in cages. Before the experiment began, they were in quarantine with a clinical examination for two weeks. The animals were kept on a balanced diet containing all the necessary biologically active and nutritious substances. They had free access to feed and drinking water. Were monitored changes in body weight and animal feed intake. The duration of the experiment was 7 days. Euthanization of animals and selection of biological material were carried out on the 8th day of the experiment.

During the experiment followed the requirements of the "European Convention for the Protection of Vertebrates used for experimental and scientific purposes" (Strasbourg, 1986), the Law of Ukraine "On the Protection of Animals against Cruel Treatment" No. 3447 of 21.02.2006.

Histomorphological Studies. After decapitation, a pathological anatomical dissection and selection of liver samples were performed for histomorphological studies. Pieces of liver were selected for microscopic studies and were fixed in a 10% solution of neutral buffered formalin [14], washed in running water, dehydrated in spirits of increasing concentration (70°, 96°), aged in chloroform, and poured into paraffin. The cooled paraffin blocks were attached to wooden cubes. After that, slices were made on a sine microtome, and slices 10 μm thick were obtained. The resulting sections were glued onto slides, stained with hematoxylin Karatsu and eosin, and examined with a light microscope [21].

Morphological and Biochemical Studies of Blood. Native blood was collected from rats from the abdominal aorta into test tubes with heparin solution, which was examined for hemoglobin content, absolute number of erythrocytes, leukocytes, and platelets, calculated by leukogram, and thrombocrit was determined on a Micro CC-20 Plus Auto HTI (USA) analyzer. To obtain plasma, it was centrifuged at 1500 rpm for 15–20 min. In blood plasma of rats were investigated biochemical indicators, such as total protein, total and conjugated bilirubin, activity of aspartate-aminotransferase (AST, EC 2.6.1.1), alanine-aminotransferase (ALT EC 2.6.1.2), γ-glutamyl-transpeptidase (γ-GTP, EC 2.3.2.2), alkaline phosphatase (APF, EC 3.1.3.1) on an open biochemical semi-automatic analyzer GBG Stat Fax 1904 Plus (Awareness Technology, Inc., Florida, USA) using DAC-SPECTROMED SRL (Moldova) reagents.

Statistics. The results were processed in the package Statistica 6.0. The probability of the difference between the samples was estimated by Student's t test, having previously checked the normality of their distribution. The disagreements were considered reliable at $P < 0.05$.

2.2 Results and Discussion

Regular administration of tetracycline hydrochloride preparation to rats for seven days is accompanied by the emergence of severe clinical symptoms of acute toxic dystrophy of liver that begin to appear in animals on the second or third day of its administration and are characterized by general depression, decreased appetite, polydipsia or thirst reduction, losses the average body weight for the group of 10–15 g, a dull coat, a decrease in elasticity and dry skin, hair loss, a slight increase stomach, liquefaction of stool.

Pathoanatomical Studies. Data from the clinical manifestation of toxic hepatodystrophy complement the results of pathoanatomical studies of the body—the liver acquires a light yellow color or has a mottled mosaic pattern, the brownish-red areas alternate with yellow, slightly enlarged, rounded edges, flabby consistency.

Histomorphological Studies. Histomorphological examination shows the decomposition of hepatocytes, diffuse placement of fat droplets of different sizes (small, medium, and large droplets) in liver cells, focal histiolymphoid infiltration, expansion, and blood vessel overflow (Fig. 1). In addition to obesity, a pronounced granular dystrophy is revealed. In some places, there are small proliferates of Kupffer cells. Changes in portal tracts are expressed in sclerosis with a slight thickening and inflammatory response—the appearance of small, local histiocytic, and lymphoid elements [22].

Morphological Indicators of Blood. As a result of the study of the morphological composition of the blood (Table 1), the development of leukocytosis (27% increase in the number of white blood cells), erythrocytopenia (a decrease in the number of erythrocytes by 27%), and simultaneous manifestation of hypochromaemia (a decrease in hemoglobin by 23%) was established in rats of the experimental group.

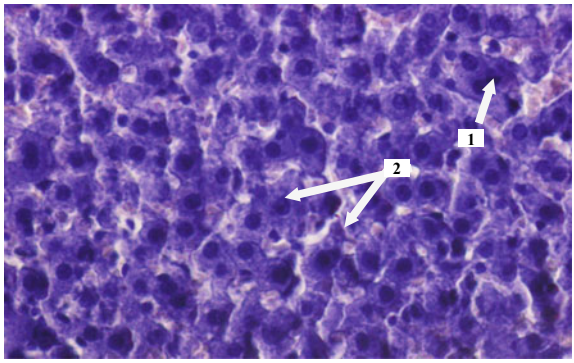

Fig. 1 Liver of the rat of study group (a dose of tetracycline hydrochloride 250 μg/kg). Hepatocytes in the state of fatty degeneration (1), hyperemia of the vessels of the particle (2). Stained with hematoxylin Karatsu and eosin, ×200

Table 1 Morphological blood indices of experimental hepatodystrophy rats against tetracycline hydrochloride at a dose of 250 mg/kg ($M \pm m$, $n = 20$, $P < 0.05$)

Index	Control group	Study group
Leukocytes, 10^9/l	4.8 ± 0.2	6.1 ± 0.5*
Erythrocytes, 10^{12}/l	6.6 ± 0.5	4.8 ± 0.3*
Hemoglobin (g/l)	182.7 ± 12.1	141.0 ± 3.6*
Mean hemoglobin in erythrocyte	28.3 ± 1.1	29.5 ± 1.7
Hematocrit (%)	35.2 ± 2.3	27.2 ± 1.7*
Platelets, 10^9/l	470.0 ± 18.0	515.0 ± 21.0
Thrombocrit (%)	0.249 ± 0.018	0.322 ± 0.016*
Lymphocytes (%)	54.0 ± 1.1	52.0 ± 1.1
Stab neutrophils (%)	8.0 ± 0.2	5.5 ± 0.5*
Segmented neutrophils (%)	33.5 ± 0.8	36.0 ± 0.4*
Monocytes (%)	2.5 ± 0.1	2.5 ± 0.1
Eosinophils (%)	1.5 ± 0.2	3.5 ± 0.5*
Basophils (%)	0.5 ± 0.0	0.5 ± 0.1

* results were significant for $p \leq 0.05$ when compared to control meanings

At the same time, the average hemoglobin content in the erythrocyte remains unchanged; it may be a compensatory response to the development of anemia, which we also observed when rats were administered toxic doses of diclofenac sodium [8]. Simultaneous reduction of the hematocrit value by 1.3 times is probably a consequence of a significant decrease in the number of erythrocytes ($P < 0.05$) and consequently proves the presence of anemia in patients with rats. The reason for increasing the value of thrombocrit (by 1.3 times), first of all, may be a violation in the hematopoiesis system or the result of the body's reaction to the development of other pathological processes that lead to stimulation of platelet production and change the parameters of the state of the platelet unit. We have diagnosed changes in

Table 2 Biochemical indicators of blood plasma in rats of experimental tetracycline-induced hepatosis ($M \pm m$, $n = 20$, $P < 0.05$)

Index	Control group	Study group
Total protein (g/l)	68.0 ± 0.1	60.5 ± 0.1*
Total bilirubin (μmol/l)	3.2 ± 0.1	45.6 ± 2.9*
Conjugated bilirubin (μmol/l)	1.45 ± 0.23	27.33 ± 0.81*
Alanine aminotransferase (U/l)	24.12 ± 3.31	72.05 ± 2.38*
Aspartate aminotransferase (U/l)	71.25 ± 3.11	126.83 ± 4.04*
Alkaline phosphatase (U/l)	250.02 ± 10.02	400.10 ± 9.79*
γ-Glutamyl transpeptidase (U/l)	12.32 ± 0.68	21.34 ± 1.10*

* results were significant for $p \leq 0.05$ when compared to control meanings

thrombocrit in the body of animals suffering from toxic hepatodystrophy, probably due to the development of an acute inflammatory reaction in the liver parenchyma, which is confirmed by the presence of leukocytosis in these animals. In the leukogram of sick animals, a significant increase in the relative number of segmented neutrophils in 1.1 times is observed, along with a decrease in the number of stab nuclear (1.5 times), which indicates a shift of the nucleus of neutrophils to the right and, above all, is the result of toxic effects of tetracycline hydrochloride on the bone marrow.

For these animals, an increase in the amount of eosinophils in the blood is 2.3 times, which is explained by their known antitoxic function. They adsorb toxic products of protein nature and destroy them, and in areas of inflammation phagocytic immune complexes, products of tissue decay, although their phagocytic activity is lower than neutrophils.

Biochemical Indicators of Blood. In the study of biochemical indicators of blood plasma in patients with toxic hepatodystrophy in rats (Table 2), there is a significant increase in activity of liver-specific enzymes: alanine aminotransferase 3 times, aspartate aminotransferase—81%, alkaline phosphatase—60% and γ-glutamyl transpeptidase 73% with a simultaneous decrease in the total protein content by 11%, an increase in the concentration of total bilirubin by 15 times due to the conjugated fraction, the level of which increased by 19 times compared to the control, indicating destruction changes in hepatocytes, a decrease in the intensity of protein-synthesizing processes, a violation of pigment metabolism, and the development of intrahepatic cholestasis.

Thus, we proposed a method for modeling the acute form of toxic drug-induced hepatodystrophy in laboratory rats with pronounced clinical, pathologic-anatomical, and biochemical changes in both the liver and the level of the whole organism.

This experimental model can be used, first of all, in medicine and veterinary medicine in order to determine the peculiarities of ultrastructural and metabolic changes in the animal organism in the development of toxic liver dystrophy, as well as for the clinical testing of hepatoprotective profile preparations again and introduction of effective treatment regimens in animal husbandry with a similar manifestation spontaneous hepatopathology. The most important result of the development of experimental hepatodystrophy is the obtaining of desired changes in the liver parenchyma with relatively moderate clinical course in rats and their 100% survival rate.

3 Conclusions

3.1 Tetracycline-Induced Hepatosis

As a result of intragastric administration of 4% tetracycline hydrochloride solution to laboratory rats using a probe at a dose of 250 mg/kg once a day, for 7 days a complex of symptoms characteristic for acute toxic dystrophy of liver with an average severity of clinical course and absence lethal cases were marked. Regular administration of tetracycline hydrochloride preparation to rats for seven days is accompanied by the emergence of severe clinical symptoms of acute toxic dystrophy of liver that begin to appear in animals on the second or third day of its administration and are characterized by general depression, decreased appetite, polydipsia or thirst reduction, losses the average body weight for the group of 10–15 g, a dull coat, a decrease in elasticity and dry skin, hair loss, a slight increase stomach, liquefaction of stool.

3.2 Pathological Changes in the Liver

In experimental hepatodystrophy—the liver acquires a light yellow color or has a mottled mosaic pattern, brownish-red areas alternate with yellow, slightly enlarged, rounded edges, flabby consistency. Histomorphological examination shows decomposition of hepatocytes, diffuse placement of fat droplets of different sizes in liver cells, focal histophilic infiltration, expansion, and overflow with blood vessels. In addition to obesity, a pronounced granular dystrophy is revealed.

3.3 Morphological Indicators of Blood

For patients with toxic hepatodystrophy in rats, the development of leukocytosis, erythrocytopenia with simultaneous manifestation of hypochromaemia, a decrease

in the hematocrit size and growth of thrombocrit is characteristic, which indicates that these animals have an inflammatory process and anemia. In the leukogram of sick animals, there is a shift of the nucleus of neutrophils to the right, which, above all, is the result of toxic effects of tetracycline hydrochloride on the bone marrow. For these animals is characteristic eosinophilia, which is explained by their antitoxic function of eosinophils.

3.4 Biochemical Indicators of Blood

For the biochemical profile of the blood plasma of patients with toxic hepatodystrophy in rats, an increase in activity of enzymes relatively specific for the liver is characteristic: aminotransferases, alkaline phosphatase and γ-glutamyl transpeptidase and hypoproteinemia, hyperbilirubinemia due to the conjugated fraction, which indicates destructive changes in hepatocytes, a decrease in the intensity of protein-synthesizing processes, violation pigment metabolism, and development of intrahepatic cholestasis.

Finding. A method for modeling the acute form of toxic medicamentous hepatodystrophy has been developed. It can be used in experimental medicine and veterinary medicine to determine the peculiarities of ultrastructural and metabolic changes in the organism of animals with a similar spontaneous pathology of the liver, as well as for the clinical testing of hepatoprotective profile preparations again.

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