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BIOCHEMISTRY, BIOPHYSICS AND MOLECULAR BIOLOGY

The Influence of the Xymedon Preparation (Hydroxyethyldimethyldihydropyrimidine) on the Rat Liver Recovery under Toxic Damage Induced by Carbon Tetrachloride

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Preparation Xymedon (1-(ß-hydroxyethyl)-4,6-dimethyl-1,2-dihydro-2-oxopyrimidon, registration no. LS-000045), which is produced in Russia, is known as a medicine with a marked regenerative and reparative effect [1]. It is highly effectively used in clinical practice for the treatment of burns and wound healing in the postoperative period. The results of clinical studies obtained in recent years have revealed new indications for the use of Xymedon: scleroderma [2], psoriasis [3], obstructive pulmonary disease [4], gastric and duodenal ulcer [5], osteomyelitis [6, 7], and pyoinflammatory disease [8]. It was shown that Xymedon affects the key biochemical processes at the cellular and subcellular levels by activating adenylyl cyclase, which leads to a rapid accumulation of cAMP in the cell and stimulation of metabolism (primarily protein biosynthesis).

This drug also affects the system of regulation of the active transport of calcium in the cell, tissue respiration, lipid peroxidation, and antioxidant system activity [9].

Since noninfectious and infectious liver diseases in recent years are becoming of a great social importance and the modern pharmaceutical market of hepatoprotectants lacks both domestic or imported medicines that fully meet all the requirements for this pharmacological group of drugs [10, 11], the search for effective hepatoprotectants is a relevant task. More than half of the existing drugs used for the treatment of liver diseases are of a plant origin, the most common of them are based on milk thistle [12]. Taking into account the published data about the drug Xymedon and our earlier data on the reduction of signs of liver injury by carbon tetrachloride at a prophylactic administration of Xymedon [13], it can be assumed that this drug will also be effective in the regenerative therapy of the liver.

Thus, the aim of this study was to investigate the therapeutic efficacy of Xymedon in the model of a toxic liver injury induced by CCl_4.

This study was performed with 111 outbred adult rats of both sexes. To induce toxic liver injury [14], the animals were administered with carbon tetrachloride CCl_4 (diluted at a 1 : 1 ratio with vegetable oil) either subcutaneously for 3–4 days at a dose of 2 mL/kg (Scheme 1) or once orally at a dose of 4 mL/kg (Scheme 2). To therapeutic treatment, rats of the experimental group starting from the next day after the toxic liver injury induction according to scheme 1, in the first experiment were administered with Xymedon for 5 days at doses of 20, 45, and 70 mg/kg. In the second experiment, the animals received Xymedon for 19 days at doses of 5, 10, and 50 mg/kg. The animals were withdrawn from the experiment on day 10 and 20 to take blood and histological material. After CCl_4 administration according to Scheme 2, animals in the third experiment were administered with Xymedon for 3 days at doses 10 and 50 mg/kg. The control group did not receive Xymedon. At the end of drug administration, rats received ether anesthesia in compliance with the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [15]. Then, their blood was taken for biochemical studies and their liver was taken for evaluation of morphological changes. Blood was assayed for serum alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) with an RX Daytona analyzer (Randox, United Kingdom), and the De Ritis ratio (AST/ALT) was calculated. The morphological changes in the liver were studied by histological analysis. Liver samples were fixed with 10% formalin, embedded in paraffin, and stained with hematoxylin-eosin. Photomicrography were performed using a system consisting of an AxioImager M2 microscope, an AxioCamHRc digital camera (Zeiss, Germany), and a

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Biochemical blood parameters in rats

Group of rats	ALT, unit/L	AST, unit/L	De Ritis ratio	ALP, unit/L	*n*
Intact	37.38 ± 2.69	148.06 ± 8.60	4.45 ± 0.29	311.5 ± 25.0	44
Parameters in experiment 1 (induction of liver injury according to Scheme 1)					
Control, day 2	131.49 ± 22.43 $p < 0.001$	238.08 ± 15.27 $p < 0.001$	2.39 ± 0.51 $p < 0.05$	433.2 ± 65.2 $p < 0.05$	14
Control, day 6	89.13 ± 13.97 $p < 0.001$	168.52 ± 14.62	2.37 ± 0.32 $p < 0.001$	349.1 ± 26.4	13
Xymedon, 20 mg/kg, day 6	69.25 ± 19.63 $p < 0.01$	126.11 ± 22.96	2.27 ± 0.89 $p < 0.05$	430.2 ± 84.1	3
Xymedon 45 mg/kg, day 6	79.01 ± 15.54 $p < 0.001$	185.06 ± 30.79	2.39± 0.11 $p < 0.05$	476.9 ± 135.5	4
Xymedon 70 mg/kg, day 6	77.00 ± 13.81 $p < 0.001$	217.06 ± 8.92 $p < 0.05$	3.00 ± 0.50	347.1 ± 28.9	3
Parameters in the 2nd experiment (induction of liver injury according to Scheme 2)					
Control, day 4	135.69 ± 25.27 $p < 0.001$	221.20 ± 35.96 $p < 0.05$	1.73 ± 0.25 $p < 0.01$	453.1 ± 84.1	4
Xymedon 10 mg/kg, day 4	99.22 ± 17.56 $p < 0.001$	209.86 ± 14.63	2.16 ± 0.23	401.4 ± 50.2	3
Xymedon 50 mg/kg, day 4	42.77 ± 14.01	149.57 ± 8.48	3.99 ± 1.50	136.3 ± 31.3	3

Data are represented as $M \pm m$ (n is the number of animals in the group); the significance of differences from the parameters of intact rats is shown.

personal computer. The simultaneously determined parameters of intact animals were taken as a standard.

The resulting digital material was processed with Origin 6.1 software; samples were compared using Student's *t* test.

The study of the state of animals on the next day after the end of CCl_4 administration according to Scheme 1 showed a significant increase in ALT, AST, and ALP and a decrease in the De Ritis ratio compared to the intact animals (table). The activity of ALT increase most significantly (3.6 times). The revealed changes in blood biochemical parameters as well as the results of the study of histological sections of the liver of rats exposed to CCl_4 according to Scheme 1 confirmed the development of a toxic liver injury in animals.

The study of the state of rats in the control group in the dynamics showed that, on day 6, the difference from the norm (in intact rats) of the studied parameters decreased compared to the changes detected immediately after exposure to CCl_4: the level of ALT remained elevated and the De Ritis ratio remained decreased, whereas the levels of AST and ALP returned to the norm. The changes in blood detected in the control group on day 6 showed continuing destructive disorders in the liver (table).

The results of the study of biochemical parameters of blood on days 10 and 20 after the exposure to CCl_4 showed no significant difference in the control group compared to the intact animals. This fact indicates the relief of the pathological process of hepatocyte destruction due to natural compensatory responses of the body. For this reason, the values of blood biochemical parameters at the later stages of research were not included in table.

When Xymedon was administered according to Scheme 1, we detected no significant reduction in ALT in the blood plasma compared to the control values at all studied doses of the drug, which indicates a decrease in the destructive changes and an improvement of the functional state of the liver (table). On days 10 and 20 after CCl_4 administration, the biochemical parameters in the experimental groups of rats that received Xymedon, similarly to the control, did not change from the corresponding values of the intact animals.

The study of the liver histology confirmed the reduction in the pathological changes and accelerated cell regeneration parenchymal organ under the influence Xymedon. On histological specimens of the control group of rats (Fig. 1) in the experiment according to Scheme 1, hydropic and granular dystrophy with a dropping fatty infiltration of the cytoplasm and disturbance of the lobed structure with small areas of fatty degeneration (steatosis) were observed on day 6. In respective experimental groups, the expression of degenerative changes and disturbances in the structural organization were less pronounced (Fig. 2).

In the experiment performed according to Scheme 2, steatosis with injured regions located near the center of hepatic lobules around the central blood vessels were observed in the control group on day 10 (Fig. 3).

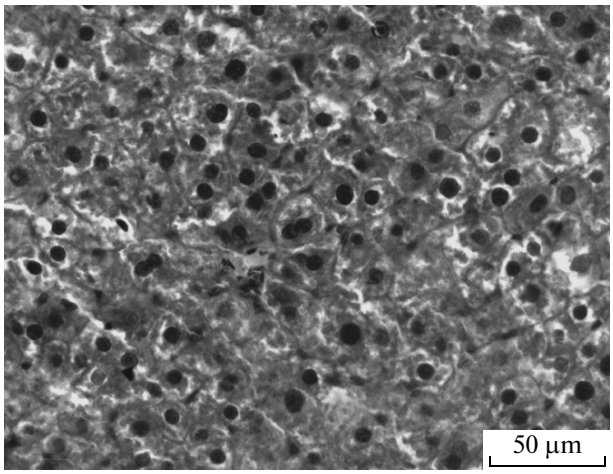

Fig. 1. Degenerative changes in hepatocytes and disruption of the structural organization of the liver in the rat of the control group on day 6 after CCl_4 administration according to Scheme 1. Here and in Fig. 2, magnification ×600.

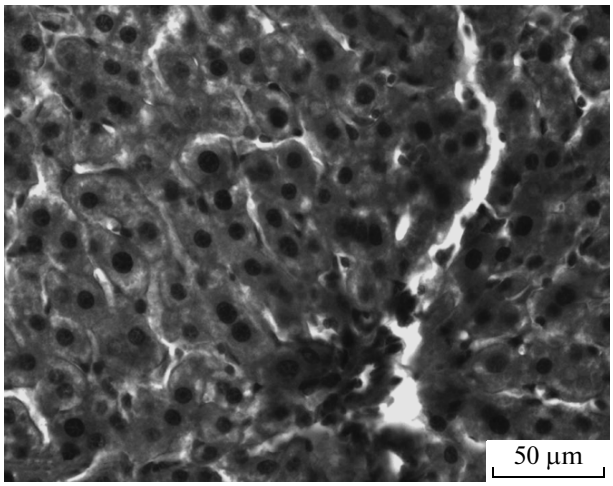

Fig. 2. Reduction in the degenerative changes in hepatocytes and normalization of the structural organization of the liver in the group of rats that received Xymedon at a dose of 20 mg/kg for 5 days after the CCl_4 administration according to Scheme 1.

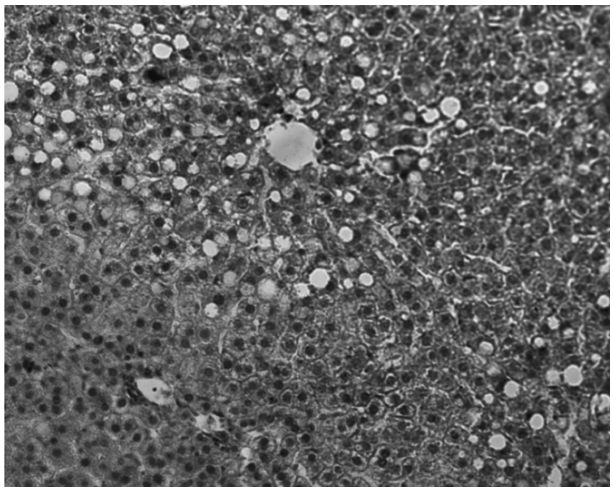

Fig. 3. Liver area with steatosis in the rats of the control group on day 10 after the CCl_4 administration according to Scheme 1. Here and in Fig. 4, magnification ×300.

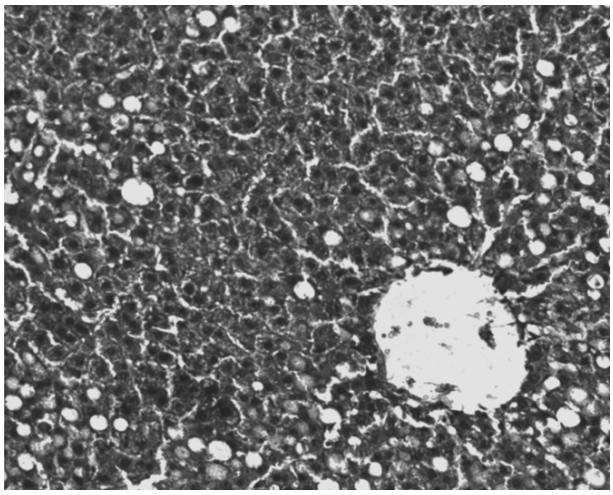

Fig. 4. Reduction in the steatosis area in the rats that received Xymedon at a dose of 5 mg/kg for 9 days after the CCl_4 administration according to Scheme 1.

The determination of the area of the normal, pathologically unchanged tissue relative to the entire area of the histological section in the field of view of the microscope in the control group on day 10 after exposure to CCl_4 showed that the proportion of healthy tissue was only 65.4 ± 2.9% (Fig. 2). This parameter did not differ from the corresponding values on the second day after the exposure to CCl_4, which accounted for 63.3 ± 2.1%. That is, in the control group, natural recover of the liver tissue did not occur during the 10-day period. On day 20, the proportion of normal, unchanged tissue in the control was 80.0 ± 4.1%.

In the animals of the experimental groups in the experiment performed according to Scheme 2, the proportion of healthy liver tissue on histological sections on day 10 was 87.6 ± 4.0, 77.6 ± 6.1, and 77.1 ± 3.0% at Xymedon doses of 5, 10, and 50 mg/kg, respectively (Fig. 4). These values were significantly higher than in the control group ($p < 0.001$, $p < 0.05$, and $p < 0.001$, respectively). On day 20, this parameter in the experimental groups was 97.60 ± 0.50, 86.5 ± 3.2, and 98.2 ± 0.8% when Xymedon was administered in doses of 5, 10, and 50 mg/kg, respectively (differences with the control were statistically significant at $p < 0.001$). The findings suggest that Xymedon stimulates the regeneration of the liver tissue in toxic liver injury.

In experiments performed according to Scheme 2, changes in the biochemical parameters in the control

group on day 4 after the CCl_4 administration were similar to those observed in the case of Scheme 1 (table), indicating the development of a toxic liver injury.

In the experimental groups, the deviation in the biochemical parameters from the intact animals decreased as early as on day 4 after the CCl_4 administration. At a Xymedon dose of 10 mg/kg, differences in parameters (ALT and AST levels) compared to the intact animals were observed, whereas in the group that received 50 mg/kg Xymedon, all parameter restored to the norm (table). In the experiments performed according to Scheme 2, the pathological changes in the histological structure of the liver were also reduced.

The study revealed a positive effect of the drug Xymedon on the recovery of rat liver after its toxic injury. When hepatotoxicity was induced by a single oral administration of CCl_4, Xymedon at a dose of 50 mg/kg normalizes blood biochemistry; under a more severe impact of CCl_4 (3 or 4 subcutaneous injections), only a trend to normalization was observed. The most significant improvement in the liver tissue regeneration was observed when Xymedon was administered at doses of 5–20 mg/kg.

The results of this study indicate the prospects of using Xymedon not only as a preventive drug [13] but also as a remedy for toxic liver injuries.

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