ORIGINAL ARTICLE

Analysis of Combined Treatment with Azathioprine and Thiotriazoline on the Course of Chronic Liver Disease in Male Rats

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Abstract

Aim and Scope: Hepatotoxicity of medications is an urgent problem all over the world. Such drugs are particularly dangerous during chronic liver disease. Therefore, today relevant research is devoted to the problem of hepatotoxicity of drugs, and to finding means for its prevention. The purpose of the study was to analyze the combined effect of azathioprine and thiotriazoline on the course of chronic liver disease with the presence of hepatocellular insufficiency in male rats. **Materials and Methods:** For the study, 30 sexually mature white male rats weighing 180–220 g were used, of which the control group consisted of 6 sexually mature white male rats. To model hepatocellular insufficiency, partial hepatectomy was performed. Experimental animals were divided into three groups: Groups 1–8 animals, comparison group without treatment, Groups 2–8 animals that received azathioprine for 20 days, and Groups 3–8 animals that received azathioprine and thiotriazoline for 20 days; then their blood sample was taken for investigation. **Results and Discussion:** Comparing the results of azathioprine with those of azathioprine combined with thiotriazoline, significant advantages of combined treatment were found: Normalization of levels of total bilirubin, total blood protein, cholesterol, a more significant decrease in alanine aminotransferase, and the absence of bone marrow suppression phenomena. **Conclusion:** Combined use of thiotriazoline and azathioprine in hepatocellular insufficiency prevents the development of toxic effects of azathioprine and may be recommended for use in patients with the chronic liver disease. Future studies on the use of thiotriazoline as a concurrent therapy to protect the liver during treatment with hepatotoxic drugs are recommended.

Key words: Azathioprine, chronic liver disease, hepatocellular insufficiency, thiotriazoline

INTRODUCTION

Hepatotoxicity of medications is an urgent problem all over the world. Such drugs are particularly dangerous during chronic liver disease. Along with this, the number of patients with viral, alcohol, and toxic liver damage is constantly growing, and the problem of non-alcoholic fatty liver disease has become especially important.[1,2] Therefore, today relevant research is devoted to the problem of hepatotoxicity of drugs, and to finding means for its prevention.[3,4]

It is known that azathioprine is a non-specific inhibitor of cell proliferation; its effect extends on proliferating cells. Therefore, this drug can suppress the development of fibrous tissue in the liver in chronic liver disease. Along with this, the use of azathioprine is limited due to its hepatotoxicity and inhibition of bone marrow function.[5] To reduce these side effects, we propose the use of an antioxidant and a membrane stabilizer thiotriazoline along azathioprine.[6-8]

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The purpose of the study was to analyze the combined effect of azathioprine and thiotriazoline on the course of chronic liver disease with the presence of hepatocellular insufficiency in male rats. We expected as result of the study to look for the possibility of neutralization of azathioprine side effects due to the hepatoprotective action of thiotriazoline.

MATERIALS AND METHODS

For the study, 30 sexually mature white male rats weighing 180–220 g were used, of which the control group consisted of 6 sexually mature white male rats.

To model hepatocellular insufficiency, partial hepatectomy was performed. Under etheric general anesthesia, laparotomy was performed. Then, right lateral lobe of the liver was taken out into the wound, afterward the ligature was applied to the corresponding branch of the hepatic artery. The next step was to remove the right lateral lobe of the liver. Resection area was treated cryogenically. After 7 days, a blood sample was taken for the study of peripheral blood indicators and evaluation of the levels of several biochemical compounds.

Experimental animals were divided into three groups:

- Groups 1–8 animals, comparison group without treatment, rats withdrawn from experiment 7 days after surgery, then their blood sample was taken for investigation;
- Groups 2–8 animals that received azathioprine in a daily dose of 0.045 mg/150 g bodyweight intramuscularly for 20 days, starting from the 8th day after surgery, then their blood sample was taken for investigation;
- Groups 3–8 animals that received azathioprine in daily dose of 0.045 mg/150 g bodyweight intramuscularly for 20 days and received thiotriazoline in daily dose of 25 mg/1 kg body weight of the rat intraperitoneally for 20 days, both starting from the 8th day after surgery, then their blood sample was taken for investigation.

The dosages of thiotriazoline and azathioprine were selected according to studies conducted under the supervision of Mazur.[6-8] All of the animals in each group followed the same diet of standard composition, conventional for rat feeding.

Animals were withdrawn (euthanasia) from the experiment on day 7 or on day 27 depending on what group they were included in. The study was conducted according to OECD guidelines for toxicity studies in animals.[9]

The investigation of peripheral blood cell levels and biochemical indices in experimental animals was carried out in a certified laboratory of Ternopil State Medical University.

Statistically significant differences between the results of various groups of rats were determined using the nonparametric Mann–Whitney U-test criterion with a generally accepted level of significance $P < 0.05$.

RESULTS AND DISCUSSION

After partial hepatectomy, beginning from 6 to 7th day a decreased reaction to noise stimuli, deteriorated appetite, loss of wool glitter, icteric coloration of the ear canals developed in experimental rats in comparison to the control ones.

The analysis of the results of laboratory tests carried out on the 7th day after partial hepatectomy in Group 1 revealed clear signs of hepatocellular insufficiency, namely: In the peripheral blood - a decreased level of erythrocytes compared to the control; in serum - an increase in the level of total bilirubin by 20.01% ($P < 0.05$) was observed due to its unconjugated fraction, and a decrease in the total protein content by 13.92% ($P < 0.05$) and cholesterol by 22.60% ($P < 0.05$) compared to those in control animals. Along with this, an increase in the level of transaminases was found: Alanine aminotransferase was 2.97 times ($P < 0.05$), aspartate aminotransferase was 15.34 times ($P < 0.05$) relative to the results of the control group [Table 1].

Thus, partial hepatectomy induced development of hepatocellular insufficiency in rats. On the 8th day after the surgery, animals of Groups 2 and 3 were given medications according to the above-mentioned schemes.

After 20 days of treatment animals in the Group 3 regained usual appetite, icteric coloration of the ear canals disappeared, wool changed back to its normal appearance, but the condition of the animals in Group 2 did not change significantly, although there was some improvement in appetite, disappearance of jaundice.

Table 1: Biochemical indices in study animals

Indicator	Group of rats			
	Control (*n*=6)	Group 1 (*n*=8)	Group 2 (*n*=8)	Group 3 (*n*=8)
Bilirubin total, μmol/L	31.98±4.36	38.38±6.04*	26.42±3.71	30.59±3.49
Blood protein total, g/L	71.56±3.31	61.60±3.30*	64.90±0.94*	69.49±2.63*†‡
Cholesterol, mmol/L	2.08±0.13	1.61±0.16*	3.87±1.23*†	5.36±0.81*†‡
Alanine aminotransferase, IU/L	23.45±1.39	69.55±6.34*	56.36±2.99*†	49.74±3.55*†‡
Aspartate aminotransferase, IU/L	16.01±1.21	245.63±20.92*	185.73±4.75*†	193.74±5.94*†

*Statistical significance of difference relative to control group (P<0.05), †statistical significance of difference relative to Group 1 (P<0.05), ‡statistical significance of difference relative to Group 2 (P<0.05)

In the peripheral blood of animals of the 2nd group, there was a decrease in the level of erythrocytes by 18.34% ($P < 0.05$) and the level of leukocytes was decreased by 23.49% ($P < 0.05$) relative to control group. In animals of the 3rd group levels of erythrocytes and leukocytes did not differ from control group but they differed significantly in comparison to the respective results of Group 2 ($P < 0.05$). The analysis of biochemical parameters showed that the level of total bilirubin was significantly lower in animals of Groups 2 and 3 compared with Group 1 ($P < 0.05$) and reached control values ($P > 0.05$) without an intergroup difference between Groups 2 and 3 ($P > 0.05$) [Table 1].The level of total blood protein tended to increase in Group 2 ($P > 0.05$) and was significantly increased in Group 3 ($P < 0.05$), reaching physiological reference values and so indicating a normalization of protein-synthetic liver function in Group 3 of rats. At the same time, there was a significant intergroup difference between 2nd and 3rd groups on this measure ($P < 0.05$). The increase of cholesterol levels relative to Group 1 was noted in animals of both 2nd and 3rd groups ($P < 0.05$), and this indicator in the past two groups was also significantly higher than in the control group ($P < 0.05$). There was at the same time a significant intergroup difference between Groups 2 and 3 in cholesterol levels ($P < 0.05$). The positive dynamics of transaminase levels was observed in the 2nd and 3rd groups relative to the 1st group: The alanine aminotransferase level was significantly lowered, respectively, by 19.07% ($P < 0.05$) and by 28.41% ($P < 0.05$), with the presence of an intergroup difference between Groups 2 and 3 ($P < 0.05$); the level of aspartate aminotransferase also significantly decreased, respectively, by 34.39% ($P < 0.05$) and 21.13% ($P < 0.05$), although without a significant intergroup difference between Groups 2 and 3 ($P > 0.05$).

Thus, the partial resection of the liver (right lateral lobe) in the experimental animals caused the development of hepatocellular insufficiency. The use of azathioprine for 20 days led to a decrease in the level of total bilirubin, an increase in cholesterol, indicating a positive effect on the functioning of liver tissue, along with a decrease in the level of red blood cells and leukocytes (bone marrow suppression). Comparing the results of azathioprine with those of azathioprine combined with thiotriazoline, significant advantages of combined treatment were found: Normalization of levels of total bilirubin, total blood protein, cholesterol, a more significant decrease in alanine aminotransferase, and the absence of bone marrow suppression phenomena, suggesting the use of azathioprine in combination with thiotriazoline in patients with chronic liver disease complicated by hepatocellular insufficiency. The results obtained can be explained by the fact that thiotriazoline is characterized by sufficient antioxidant properties well confirmed in other experiments and clinical settings.[8,10] In the works of Ukrainian authors, its membrane stabilizing, anti-inflammatory, anti-ischemic, reparative, and immunomodulatory effects in chronic liver disease have been proven. [11-14] This indicated mechanism of action of thiotriazoline ensures hepatoprotective effect when using with azathioprine in liver pathology with hepatocellular insufficiency.

CONCLUSION

Combined use of thiotriazoline and azathioprine in hepatocellular insufficiency prevents the development of toxic effects of azathioprine and may be recommended for use in patients with the chronic liver disease. Future studies on the use of thiotriazoline as a concurrent therapy to protect the liver during treatment with hepatotoxic drugs are recommended.

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