The Effect of the Course Intake of Hilak Mineral Water on Liver Function in Experimental Toxic Hepatitis and Intrahepatic Cholestasis

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***Abstract* – The effect of a two-week intake of bicarbonate carbon dioxide-sodium chloride mineral water of North Ossetia "Hilak" with a total mineralization of 2.1–2.3 g/l on the functional state of the liver of Wistar rats with toxic hepatitis and intrahepatic cholestasis was studied. Toxic hepatitis was modeled by triple intragastric administration of carbon tetrachloride in a dose of 1.5 ml/kg on 42 rats, and intrahepatic cholestasis also in 42 rats by a single intragastric administration of α-naphthilisothiocyanate at a dose of 100 mg/kg. The introduction of carbon tetrachloride in three days causes an increase in blood bilirubin, alkaline phosphatase and alanine aminotransferase activity, a decrease in cholesterol, hemoglobin, red blood cell count, hematocrine number and de Rytis coefficient. The two-week constant free intake of Hilak mineral water had a normalizing effect on the studied parameters. In experimental intrahepatic cholestasis, after 72 hours, there is an increase in the blood content of cholesterol, bilirubin and alkaline phosphatase. The preventive and therapeutic intake of mineral water, when rats had free access to mineral water for two weeks, then α-naphthylisothiocyanate was introduced to them and after three days they received mineral water, had the most positive effect. Conducting further research may allow recommending the intake of Hilak mineral water as a therapeutic agent for patients with liver pathology.**

Key words – hilak mineral, hepatitis, intrahepatic cholestasis, liver

I. INTRODUCTION

Currently, the use of mineral waters for the prevention and treatment of many diseases is important because they are natural therapeutic factors of multifaceted nonspecific action and can, as they are further studied, take one of the leading places in the rehabilitation and rehabilitation of the population.

The hydrocarbonate carbon dioxide – sodium chloride mineral water of North Ossetia “Hilak” (the source is located 55 km from Vladikavkaz in Kurtatinsky gorge at an altitude of 2500 m above sea level) with a total mineralization of 2.1–2.3 g/l and a high content of iron, boron and silicon, at the conclusion of the Pyatigorsk Scientific Research Institute of Health Resort and Physical Culture, is recommended for bottling as therapeutic mineral water for a number of chronic diseases of the gastrointestinal tract, kidneys and urinary tract.

After a retrospective analysis of the effect of mineral waters of similar or similar composition studied by us, improvements in patients with type 2 diabetes can be noted

(daily intake of 500 ml of mineral water in Japan reduced the level of glycated hemoglobin) [1].

Drinking low-mineralized bicarbonate sulphate-sodium-calcium carbonate water improves the characteristics of somatic and neurological status, activates metabolic processes, accelerates lymph and blood circulation [2].

The course intake of low-mineralized sodium chloride-bicarbonate-sodium water significantly improved the antitoxic function of the liver, reduced inflammation, normalized the activity of liver enzymes and stimulated the protein-synthetic function with positive dynamics of the morphological and histochemical characteristics of the liver during experimental toxic hepatitis [3].

Positive hepatoprotective effect, improvement of the protein spectrum, inhibition of transaminase activity and accumulation of malondialdehyde in the liver, improvement of excretory and antitoxic function, were obtained in tetrachloromethane toxic hepatitis rats, after taking therapeutic table hydrogen chloride carbonate sodium mineral water [4].

II. Formulation of the problem

To study the effect of the two-week intake of bicarbonate carbon dioxide-sodium chloride mineral water “Hilak” on the functional state of the liver in experimental models of toxic damage and intrahepatic cholestasis.

III. Materials and methods

The model of toxic liver damage was caused by oral administration to rats through a tube into the stomach for two consecutive days of carbon tetrachloride (CCl4) at a dose of 1.5 ml/kg body weight at a dilution of 1: 1 with olive oil. To fix the effect at the end of the week, this mixture was injected one more time.

Other authors used a similar model of acute toxic liver damage in rats and rabbits with a dose of carbon tetrachloride of 1.0 ml/kg [5, 6], but at the same time they injected the hepatotoxic mixture intraperitoneally and, instead of drinking water, gave the animals a 10 % solution of ethyl alcohol. This method often, along with the development of toxic hepatitis, contributes to the emergence of various inflammatory processes, ulcers and cirrhosis. Other authors injected rats with carbon tetrachloride in the form of a 66% oil solution at a dose of 2.0 ml/kg by 4–8 subcutaneous injections [7], and some, on the contrary, at a dose of three times less than us [8].

Experiments on the study of toxic hepatitis were carried out on 56 matured Wistar rats weighing from 170 to 230 g (188.5 ± 19.6 g), 14 of which were intact, the rest experimental, divided into 3 groups (14 each): the first group with toxic hepatitis; the second group of rats that received free water for two weeks of tap water (control) and the third group – for the same days and under the same conditions the Hilak mineral water (experimental).

The model of intrahepatic cholestasis was created by a single intragastric administration in the form of a 2% solution in olive oil of α-naphthyl isothiocyanate at a dose of 100 mg/kg [9]. In this case, the seed went through 12 hours after the removal of food, which was given to the rats after two hours. Studies were performed 72 hours after the administration of the toxicant. The choice of time for research after three days was due to the fact that it was the time of maximum manifestations of violations of the studied parameters, when some of them reached values after two days, and then began to normalize, while others changed as much as possible on the fourth day, with full normalization to the seventh day.

Experiments to study the effect of mineral water in intrahepatic cholestasis were also carried out on 56 adult Wistar rats weighing from 160 to 210 g (178.6 ± 15.5 g), 14 of which constituted the control group with cholestasis (I), 14 – the prophylactic group, which for two weeks received mineral water in a free mode, and then they were intoxicated (II), 14 rats were prophylactic and therapeutic group, that is, unlike the previous group, after the introduction of α-naphthylisothiocyanate, they continued to receive Hilak mineral water (III), and the remaining 14 rats constituted a purely medical group (IV), which were first given α-naphthylisothiocyanate, and then for three days they received Hilak.

Blood for research in rats in the state of anesthesia (intraperitoneal injection of anesthetic zoletil at a dose of 0.1 ml/100 g) was taken from the heart and plasma spectrophotometrically (UNICO-2000, USA) determined the content of hemoglobin, cholesterol, total and direct bilirubin, aspartate aminotransferase activity (AsAT) and alanine aminotransferase (AlAT) and alkaline phosphatase. The de Ritis coefficient was calculated by the ratio of the activity of serum transferase AsAT and AlAT. In rats with intrahepatic cholestasis in blood plasma, the contents of bilirubin and cholesterol, the activity of alkaline phosphatase were determined.

The obtained results were subjected to statistical processing using a parametric method of comparison of average values, the reliability of differences between the groups was evaluated by the Student's t-test.

The maintenance of rats, care, experiments and the withdrawal of animals from the experiment, followed by recycling, were carried out in accordance with the order of the Ministry of Health and Social Development of Russia No. 708n of August 23, 2010 “On Approval of the Laboratory Practice Rules”, taking into account GOST 33215-2014 and care for laboratory animals. Rules for equipment of premises and organization of procedures” from 01.07.2016

Permission for conducting experimental studies on animals for this research work was obtained from the Ethics Committee of the North Ossetian State Medical Academy, November 25, 2017, protocol No. 7.18.

IV. The discussion of the results

Blood levels of bilirubin and cholesterol, alkaline phosphatase and ALT activity are closely related to the functional state of the liver. Normally, the serum activity of AlAT and AsAT is low [10], since these enzymes are mainly intracellular (AlAT predominantly in the liver and kidneys, and AsAT in cardiomyocytes), therefore an increase in one or the other suggests that possible pathology of the liver or myocardium.

The activity of these enzymes in intact rats was 0.262 ± 0.04 µkat/L (AlAT) and 0.273 ± 0.024 µkat/L (AcAT). Three days after the introduction of carbon tetrachloride to the rats, the activity of AlAT increased 1.95 times (p<0.001), and AcAT – slightly. The two-week intake of tap water did not affect the activity of AlAT and it remained elevated (0.443 ± 0.056 µkat/L), and the intake of mineral water reduced it to less than the intact level. The activity of AsAT after two weeks of intake of tap and mineral water became less than normal (0.207 ± 0.018 µkat/L and 0.16 0 0.02 µkat/L, respectively).

The de Rytis coefficient used to determine the prevailing damage to the liver or myocardium showed that in intact rats it corresponded to the results obtained by other authors [11, 12] and was 1.04 ± 0.05. But three days after intoxication, the coefficient almost halved (p0.001). In rats with toxic hepatitis who received tap water for two weeks, the de Ritis coefficient decreased even more, and with mineral water increased to 0.73 ± 0.04 (p<0.01).

Thus, the marked increase in the blood plasma activity of ALT indicates hepatocyte damage, and the decrease in enzyme activity to the level of the norm after a two-week intake of "Hilak" is associated with its positive effect on the liver.

Another indicator of the functional state of the liver is alkaline phosphatase activity, which is found in all tissues of the body, with a predominant localization in the liver and bones, so an increase in the activity of alkaline phosphatase in the blood indicates their damage [13]. In intact rats, plasma phosphatase activity was 587.25 ± 33.98 IU/L, and three days after the last injection of carbon tetrachloride, it was statistically significantly (p<0.02) increased to 767.54 ± 38.64 IU/L. The two-week intake of Hilak contributed to a decrease in enzyme activity to 514.29 ± 40.08 IU/l (p<0.01), while in control rats it remained high (717.68 ± 40.08 mU/l). Obviously, a decrease in alkaline phosphatase activity after ingestion of mineral water could be due to a weakening of the process of lipid peroxidation, and also, possibly, the composition of mineral water induces microsomal enzymes of liver cells, increasing their formation rate and activity, or, like many other mineral waters , increasing diuresis and contributes to a more rapid elimination of toxic substances from the body [14, 15], reducing the effect of carbon tetrachloride.

Plasma cholesterol level, as one of the indicators of the functional state of the liver, in intact rats was 1.56 ± 0.07 mmol/l, and after the administration of CCl4 on the third day it decreased by 34.0 % (p0.001). A two-week intake of mineral water increased the cholesterol content to 1.29 ± 0.07 mmol/l and, although it was less than the norm (p<0.05), but at the same time it became statistically significant (p<0.05) more than it was on the third day after intoxication. Drinking tap water did not affect cholesterol levels, which remained low (1.06 ± 0.06 mmol/l). It is possible that an increase in blood cholesterol after taking Hilak may be because not only on the liver, but also on the small intestine, kidneys and other organs in which it is also synthesized.

The introduction of carbon tetrachloride caused a change in some hematological parameters. So, on the third day, the number of red blood cells, hemoglobin content and hematocrit number decreased by 27.7, 26.5 and 16.5%. Two weeks later, these indicators in rats receiving tap water remained within the limits of reduced values, and those receiving “Hilak” increased, approaching the values of intact animals.

The decrease in the number of erythrocytes and the hemoglobin content is due to the effect of CCl4 to weaken the osmotic resistance of erythrocyte membranes and the activity of enzyme systems that ensure, due to the optimal operation of Na +, K + -ATPases and Ca ++ - ATPases, the normal content of these ions inside erythrocytes [16]. With the defeat of hepatocytes, there is a violation of the deposits in the liver of protein-cobalamin [17], necessary for the formation of folinic acid involved in erythropoiesis. At the same time, carbon tetrachloride can also inhibit leuko- and thrombopoiesis [18].

It is obvious that a decrease in the number of red blood cells and an increase in the blood content of bilirubin are due to the destruction of red blood cells. The content of total bilirubin in the blood of intact rats was 2.24 ± 0.13 µmol/L (1.37 ± 0.09 µmol/L — direct bilirubin and 0.87 ± 0.06 µmol/L indirect). Three days after the third injection of carbon tetrachloride, the bilirubin and its fractions increased significantly (total 5.63 ± 0.38 µmol/l, direct 4.15 ± 0.35 µmol/l and 1.48 ± 0.09 µmol/l indirect). A two-week intake of tap water did not affect the content of bilirubin, and in rats treated with Hilak it was close to that of intact rats.

In experimental intrahepatic cholestasis, cholesterol level after α-naphthylisothiocyanate administration significantly (4.65 times) increased, reached 7.26 ± 0.56 mmol/l (in intact rats, 1.56 ± 0.07 mmol/l). A preliminary two-week prophylactic intake of mineral water contributed to the fact that the content of cholesterol, 72 hours after the introduction of the toxicant, increased to 4.12 ± 0.35 mmol/l, which was 2.98 times the intact level. And with continuation after seeding, for another three days, taking mineral water (prophylactic and therapeutic option), the increase in cholesterol in the blood plasma was only 2.25 times (3.51 ± 0.28 mmol/l). The smallest positive result in reducing plasma cholesterol was obtained in treatment group rats who received Hilak only for three days after intoxication. It remained 3.74 times more than the norm of intact animals (5.83 ± 0.41 mmol/l).

Simultaneously with the change in plasma cholesterol after single oral administration of α-naphthyl-isothiocyanate to rats, bilirubin changed from an intact level of 2.24 ± 0.13 µmol/l (total), 1.37 ± 0.09 µmol/l (straight) and 0.87 ± 0.06 µmol/l (indirect) to 86.45 ± 6.52 µmol/L, 82.84 ± 7.28 µmol/L and 3.61 ± 0.28 µmol/L, respectively. Such a significant increase in total and direct bilirubin indicates a clear violation of the free passage of bile along the biliary tract and its entry into the intestine, that is, the introduction of α-naphthylisothiocyanate contributed to the inflammation of the biliary ducts, their swelling and disruption of the outflow of bile. Preventive intake of mineral water reduced the level of total bile pigment by 2.45 times (35.28 ± 2.63 µmol/l), 2.52 times (32.87 ± 4.07 µmol/l) of its direct fraction, and indirect bilirubin was 2.41 ± 0.18 µmol/l. The more pronounced changes in bilirubin towards normalization were in rats of the III preventive treatment group (28.72 ± 1.96 µmol/L, 26.94 ± 2.64 µmol/L and 1.78 ± 0.09 µmol/L, respectively), and the smallest - in animals of the treatment group (44.62 ± 3.85 µmol/L, 41.32 ±

2.88 μmol/L and 3.30 ± 0.27 μmol/L (total bilirubin, direct and indirect).

Similar changes were applied to the activity of alkaline phosphatase, which from the control level of intact rats at 587.25 ± 33.98 IU/l after the introduction of α-naphthyl-isothiocyanate increased to 1425.0 ± 133.5 IU/l. In the rats of the prophylactic-therapeutic group, the activity was 796.25 ± 95.89 IU/l, and during the treatment, it was 1012.72 ± 89.34 IU/l. That is, the greatest positive changes were noted, as well as with cholesterol and bilirubin in rats of the prophylactic-therapeutic group.

V. CONCLUSION

1. Triple administration of carbon tetrachloride at a dose of 1.5 ml/kg body weight to Wistar rats for one week causes: an increase in blood bilirubin content, alkaline phosphatase activity and ALT; reduction of cholesterol, hemoglobin, red blood cell count, hematocrine number and de Rytis coefficient.

2. A two-week constant free intake of bicarbonate carbon dioxide-sodium-chloride mineral water “Hilak” with a total mineralization of 2.1–2.3 g/l has a normalizing effect on the blood parameters under study.

3. In experimental intrahepatic cholestasis created on Wistar rats by a single intragastric administration of a 2% solution of α-naphthylisothiocyanate at a dose of 100 mg/kg, after 72 hours there is an increase in blood cholesterol, bilirubin and alkaline phosphatase. Preventive and therapeutic intake of mineral water (free access to mineral water for two weeks, then intoxication and continued intake of mineral water for three days) has the most positive effect.

4. Conducting further research will allow recommending the intake of Hilak mineral water as a therapeutic agent for patients with liver pathology.

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