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HEPATOPROTECTIVE EFFECT OF ANTIDYSBIOTIC DRUGS IN EXPERIMENTAL METABOLIC SYNDROME

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Abstract

Modeling of experimental metabolic syndrome in Wistar male rats was accompanied by a sharp increase in neutrophils/lymphocytes ratio and decrease in the numbers of monocytes in the blood, as well as almost 5-fold increase in the urease activity, a 3-fold decrease in the activity of lysozyme, and significant increase in the levels of inflammatory markers (MDA and elastase) in the liver. A 13-fold increase was observed in the level of dysbiosis in the liver and a significant increase in the alkaline phosphatase activity. Using antidysbiotic drugs (Quertulin, Quertgial and lysozyme) normalized the state of liver.

Key words: liver, metabolic syndrome, dysbiosis, immunodeficiency, antidisbiotic drugs.

Metabolic syndrome (MS) is a pathological condition of the body, which is based on excessive consumption of high-calorie foods, and it is completely balanced for all essential nutritional factors [12, 16]. This is most often associated with excess intake of fats, especially with a high content of palmitic acid [5] or easily digestible carbohydrates (sugar, starch), which are converted into palmitic acid in the liver, and then into the corresponding

triglycerides that are formulated by the liver in very low density lipoproteins. [6]. High-calorie diets, especially high-fat diets (WGW), cause the development of obesity [20], the consequence of which is the chronic activation of the sympathetic system [14]. At the same time, there is an increase in phosphorylation, activation of hormone-dependent lipase lipase, and enhanced hydrolysis of triglycerides with an increased amount of free fatty acids entering the blood [7]. The latter cause the development of insulin resistance - the most important factor in the pathogenesis of type 2 diabetes mellitus and MS [10, 22]. A further pathogenetic link in the development of MS is changes in the species composition of the endogenous microflora and its content, which are defined as dysbiosis [9, 21, 25]. Obligatory elements of dysbiosis are endotoxemia (mainly due to lipopolysaccharide [22]) and systemic inflammation [15, 24]. The result of this can be considered a deep disturbance of lipid metabolism, manifested by hyperlipidemia and liver steatosis, turning into steatohepatitis [11, 23]. The leading role of dysbiosis in the pathogenesis of MS is confirmed by the therapeutic effect of the use of anti-disbiotic drugs [13, 18-20].

The purpose of the work is to study the effect of a number of new antidisotic drugs on the state of the liver in experimental MS.

Material and methods. The following substances were used in the work: quercetin (Merck, United States; content of the main substance 99.6%), chicory root inuline (Consucra Groupe Waxoing SA, Belgium), calcium citrate (China), hyaluronic acid (drug “Gengigel”, “Ricerpharma”, Italy), lysozyme (preparation “Clerizyma”, “Caglificio Clerici SpA”, Italy). The following drugs were prepared from these substances: • “Quertulin” (TU U 10.8-13903778-040: 2011) - contains quercetin, inulin and calcium citrate; • “Kvertgial” (TU U 20.4-13903778-032: 2012) - contains quertulin and hyaluronic acid; • “Lysozyme in gelatin” (10% Clerizyma in 10% gelatin solution). In the experiments, 35 white male rats of the Wistar line (4 months, weighing 250 g) were used, divided into 5 groups (7 each): 1 - intact, 2 - MS - dysbiosis + immunodeficiency + high-fat diet (VZHR) [17], 3 - MS + the drug “Kvertulin”, 4 - MS + the drug “Lysozyme in gelatin”, 5 - MS + the drug “Kvertgial”. Doses of drugs are listed in the table. 1.

Dysbiosis was caused by the antibiotic lincomycin, which was given with drinking water at a dose of 60 mg / kg daily for 5 days. Immunodeficiency was created by intravenous administration of cytostatic cyclophosphane at a dose of 25 mg / kg every other day. WZHR was obtained by adding to the feed 15% of unrefined sunflower oil. The duration of the experiment was 21 days. Anti-disbiotic drugs began to be administered from the first day of the experiment.

Table. 1.

Experimental groups of rats and doses of drugs

Group	Therapeutic drug	Components mg / kg
Intact	–	–
Metabolic Syndrome (MS)	–	–
MS + Querthulin	Querthulin powder 300 mg / kg per os	Quercetin - 5 Inulin - 180 Calcium Citrate — 115
MS + lysozyme	Lysozyme in gelatin 1000 mg / kg	In terms of pure lysozyme — 20
Ms + quertial	Kvertgial-gel 0.5 ml / rat application on the CAPR	Quercetin - 0.68 Inulin - 24.0 Calcium Citrate - 15.32 Hyaluronic Acid — 0,8

Euthanasia of animals was performed on the 22nd day under thiopental anesthesia (20 mg / kg) by total bleeding from the heart. The blood composition of leukocytes was determined in the blood [4]. In the liver homogenate (50 mg / ml 0.05 M Tris-HCl buffer pH 7.5), the activity of urease (microbial contamination index) [3], lysozyme (non-specific immunity indicator) [3], the level of inflammation markers [10] were determined: malonic dialdehyde (MDA), elastase activity, the antioxidant enzyme catalase and alkaline phosphatase (alkaline phosphatase, cholestasis marker) [2].

The ratio of the relative activities of urease and lysozyme was calculated enzymatic indicator of the degree of dysbiosis [3]. According to the ratio of catalase activity and the content of MDA, the antioxidant-prooxidant index API was calculated [2]. Statistical processing of the results was carried out in accordance with the instructions [1].

Results and its discussion.

The results of determining the cellular composition of the blood of rats with experimental MS who received anti-disbiotic drugs are presented in Table. 2.

From these data it can be seen that the content of leukocytes in MS decreases slightly, and mainly due to a significant decrease in the number of lymphocytes, as evidenced by the multiple increase in the ratio of neutrophils / lymphocytes. The number of monocytes decreases almost 2 times.

Table 2

Effect of quertulin, lysozyme and quergial on the cellular composition of the blood of rats with MS, M ± m

Group	Leukocyte, $\cdot 10^9$ /l	Neutrophils / Lymphocytes	Monocytes, %
Intact	12,8 ± 1,5	0,37 ± 0,03	10,4 ± 0,5
Metabolic Syndrome (MS)	9,9 ± 1,2	3,16 ± 0,20***	5,6 ± 1,3**
MS + Querthulin	11,8 ± 1,6	3,24 ± 0,25***	5,8 ± 0,7**
MS + lysozyme	7,6 ± 1,1*	2,42 ± 0,13***#	9,4 ± 1,0#
Ms + quertial	11,6 ± 0,9	1,97 ± 0,12***##	7,8 ± 0,8*

Notes (here and in Tables 3-5): * - P <0.05, ** - P <0.01, *** - P <0.001 compared to intact ones; # - P <0.05, ## - P <0.01 compared with MS.

The use of antidisbiotic drugs has a different effect on the cellular composition: lysozyme and quertial reduce the neutrophil / lymphocyte ratio, but do not return it to normal and increase the number of monocytes, but significantly - only in the MS + lysozyme group. Unlike lysozyme and quergial, quertulin has little effect on blood counts. The results obtained by us indicate a weakening of the lymphocytic link of cellular immunity in experimental MS. Of the three tested anti-disbiotic drugs, quergial was the most effective. The results of determining the activity of urease and lysozyme in the liver of rats with MS who received anti-dysbiotic drugs are presented in Table. 3. These data show that in case of pathology without treatment, the activity of urease increases almost 5 times, which indicates an increase in the microbial contamination of the liver. On the contrary, the activity of lysozyme is reduced by 2.7 times. Anti-disbiotic drugs reduce the activity of urease and increase the activity of lysozyme. However, the activity of urease is significantly reduced only by the action of lysozyme.

Table 3

Effect of quertulin, lysozyme and quergial on the activity of urease and lysozyme in the liver of rats with MS, M ± m

Group	Urease, micron-cat / kg	Lysozyme, units / kg
Intact	0,20 ± 0,05	167 ± 15
Metabolic Syndrome (MS)	0,97 ± 0,11**	062 ± 10**
MS + Querthulin	0,73 ± 0,10**	093 ± 9**#
MS + lysozyme	0,56 ± 0,09*#	136 ± 15#
Ms + quertial	0,67 ± 0,11**	99 ± 10*#

It was shown that with experimental MS, the degree of dysbiosis in the rat liver increases 13 times (Fig. 1). All antidisbiotic drugs reliably reduce the degree of dysbiosis, although they do not return it to normal, with lysozyme and quergial being the most effective.

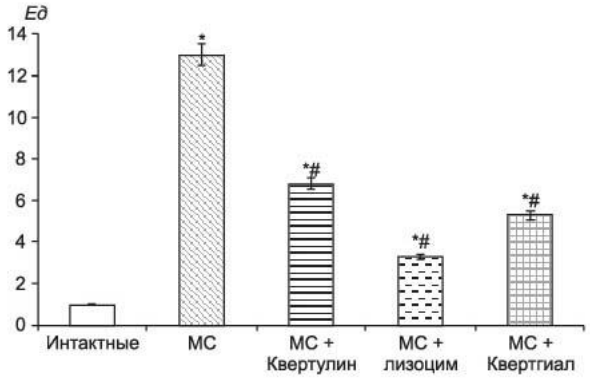

Fig. 1. Effect of Querthulin, Lysozyme and Quertihal on the degree of dysbiosis in the liver in MS. * - P <0.05 compared with intact, # - P <0.05 compared with MS.

The results of determining the level of markers of inflammation in the liver of rats with MS who received anti-disbiotic drugs are presented in Table. 4. As can be seen from these data, in pathology, the level of both markers reliably increases, and their antidisbiotic drugs reduce their level, and querculin - almost to the norm. The lysozyme drug had the weakest effect. The results of determining the activity of catalase and alkaline phosphatase in the liver of rats, which, against the background of MS, received antidisbiotic drugs, are presented in Table. 5. From these data it can be seen that the activity of liver catalase does not change either in pathology or in the presence of anti-disbiotic agents. At the same time, the activity of alkaline phosphatase significantly increases with pathology, which indicates the development of cholestasis, and significantly decreases (almost to normal) under the influence of antidisbiotic drugs, of which quergial was the most effective.

Table 4

Effect of quertulin, lysozyme and quergial on the level of inflammatory markers in the liver of rats with MS, M ± m

Group	MDA, mmol / kg	Elastase, MK-CAT / kg
Intact	37,6 ± 1,8	382 ± 8
Metabolic Syndrome (MS)	50,7 ± 2,2***	486 ± 12***
MS + Querthulin	42,0 ± 2,5#	406 ± 17##
MS + lysozyme	44,7 ± 2,4*	443 ± 22*
Ms + quertial	43,2 ± 2,9#	427 ± 13**#

Table 5

Effect of quertulin, lysozyme and quergial on the activity of catalase and alkaline phosphatase in the liver of rats with MS, M ± m

Group	Catalase, mkat / kg	ALP, mk-cat / kg
Intact	5,93 ± 0,20	4,84 ± 0,25
Metabolic Syndrome (MS)	5,78 ± 0,09	6,44 ± 0,48*
MS + Querthulin	5,86 ± 0,08	5,25 ± 0,41
MS + lysozyme	5,79 ± 0,21	5,14 ± 0,22#
Ms + quertial	5,84 ± 0,04	4,97 ± 0,12##

It was shown that the API index in the liver significantly decreases in pathology, however, it significantly increases under the influence of antidisbiotic drugs, especially those containing quercetin (Fig. 2).

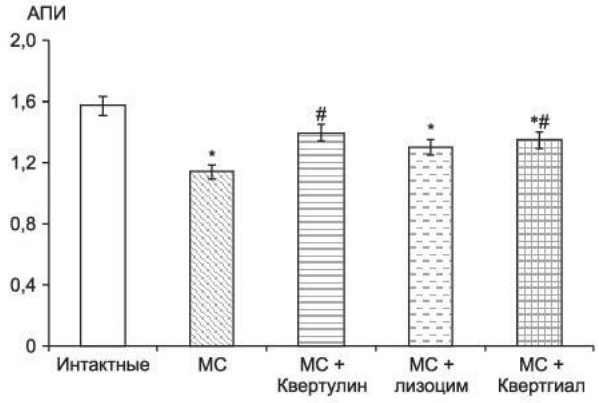

Fig. 2. Effect of Querthulin, Lysozyme and Quertihal on the amount of API in the liver with MS. * - P <0.05 compared with intact, # - P <0.05 compared with MS.

Thus, on the background of CGD, serious liver disorders are observed in experimental dysbiotic pathology complicated by immunodeficiency. Anti-disbiotic drugs have a hepatoprotective effect, which confirms the role of intestinal dysbiosis in the pathogenesis of hepatitis and dictates the primary need to eliminate intestinal dysbiosis for the prevention and treatment of hepato-biliary pathology. For this purpose, a lysozyme preparation in gelatin, which can be used orally, is more preferable. As for the hepatoprotective effect of two quercetin-containing drugs, preference should be given to quergial, which, when applied to the oral mucosa, causes the same effect as quertulin, but only in much smaller doses.

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