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THE ROLE OF INTESTINAL DYSBIOZIS IN INFRINGEMENT OF THE FUNCTION OF THE LIVER OF RATS AFTER ANTIBIOTIC THERAPY

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Abstract

In experimental work on rats was shown that antibiotics with the most pronounced hepatotoxic effects contribute more to the development of dysbiosis of the colon and to an increase in serum urease activity. On the basis of the results obtained, an assumption was made of the hepatotoxic effect of urease on the pathogenic microbiota of the intestine.

Key words: antibiotics, dysbiosis of intestinal, urease, liver

Antibiotic therapy is an integral part of modern treatment regimens for numerous diseases, but unfortunately, with a number of sides toxic and allergic effects [1]. The negative consequences of antibiotics include the development of intestinal dysbiosis, which in case of liver dysfunction extends to other organs [2]. Enhanced contamination by pathogenic bacteria with a simultaneous decrease in the number of probiotic microbiota in dysbiosis can be registered with the help of urease, the production of with is a marker of a pathogenic microorganisms. Urease acts as activator of cells of the monocyte- macrophage series, using a

mechanism that is independent of the lipopolysaccharides of bacteria and immune modulator of inflammatory reactions, induces expression of interleukin-2, interleukin-8 and tumor necrosis factor. In addition, ammonia formed under the action of urease is able to initiate generation of superoxide anion and singlet oxygen radicals by neutrophils, resulting in "oxygen explosion" in tissues and organs [3].

Undoubtedly, the aggressive urease of pathogenic intestinal bacteria, which enters the bloodstream in the liver, can not but affect the functional characteristics of this detoxifying organ. But currently, the question of the role of urease in liver dysfunction after taking antibiotics is not sufficiently covered.

The stated purpose of the work was to investigate the dependence of the functional parameters of the rat liver on the degree of intestinal dysbiosis and urease activity after taking antibiotics.

Materials and methods

Two series of experimental studies on Wistar rats were carried out. In 1th series 40 males of monthly age with an average weight of 40 ± 4,5 g were used, distributed into 5 equal groups: intact and 4 groups receiving various antibiotics (table 1).

Table 1

Characteristics and doses of antibiotics

Preparation	Group	Dose, mg/kg	Manufacturer, country
1th series			
Cefix	Cephalosporin III generation	20	«International», Jordan
Sumamed	Macrolides-azalid (azithromycin)	25	«Pliva», Croatia
Amoxyclav	Penicillin (amoxicillin) + inhibitor of β-lactamases (clavulanic acid)	40	«Sandoz», Switzerland
Lincomycin	Lincosamides	60	"Darnitsa", Ukraine
2nd series			
Omeprazole	H-K-ATPase inhibitor	1,33	«Pharmak», Ukraine
Amoxil	Beta-lactam antibiotic	50	«Kyivmedpreparat», Ukraine
Clarithromycin	Macrolides, lincosamides	7,5	«Kyivmedpreparat», Ukraine

Studies in the 2nd series was performed on 18 females (10 months, average weight 300 g), divided into 2 groups: 1st – control, 2nd received H. pylori complex, prescribed for infection of *Helicobacter pylori* (Omeprazole of 1,33 mg/kg, Amoxil 50 mg/kg and Clarithromycin 7,5 mg/kg).

Dosage and duration of administration of antibiotics are calculated in accordance with the recommendations of the developers. Antibiotics were administered with drinking water, taking into account the dose and amount of water consumed for 5 days in 1th series and 8 days in 2nd series.

All manipulations with rats were performed in a gentle mode, without exposing them to stress and pain [4]. Euthanasia of animals was carried out under thiopental anesthesia (40 mg/kg) 5 days after the last antibiotic administration by total bloodletting from the heart. Blood was collected from which serum was obtained, as well as liver and mucous layer of the colon were isolated. Serum alanine aminotransferase (ALT) activity [5], alkaline phosphatase [5], elastase [6] and urease [7], and triglyceride content [5] were determined. Urease and lysozyme activity was determined in liver homogenates and colon mucosa layer (50 mg/ml 0,05 M Tris-HCl pH 7,6), their level was used to calculate the degree of dysbiosis in Levitsky [7]. Statistical processing of the obtained results was performed using Student's t-test [8].

Results and discussion

The results presented in table 2 show a significant increase in urease activity in all the studied objects after taking antibiotics.

Table 2

Urease activity in the colon, blood serum and liver of rats after antibiotic administration

Groups of rats	Mucous layer of large intestine, mc-kat/kg	Blood serum, nkat/l	Liver, mc-kat/kg
1th series			
Intact	2,09 ± 0,41	0,91 ± 0,07	0,48 ± 0,05
Cefix, 20 mg/kg	3,62 ± 0,35 p < 0,02	1,48 ± 0,16 p < 0,01	0,75 ± 0,09 p < 0,05
Sumamed, 25 mg/kg	3,07 ± 0,29 p < 0,05	1,30 ± 0,18 p > 0,05	0,65 ± 0,10 p > 0,1
Amoxiclav, 40 mg/kg	2,76 ± 0,21 p > 0,05	1,08 ± 0,09 p > 0,05	0,49 ± 0,05 p > 0,2
Lincomycin, 60 mg/kg	4,52 ± 0,37 p < 0,001	1,67 ± 0,11 p < 0,001	0,76 ± 0,11 p < 0,05
2nd series			
Intact	1,76 ± 0,14	0,74 ± 0,06	0,21 ± 0,02
Omeprazole, 1,3 mg/kg Amoxyl, 50 mg/kg Clarithromycin,7,5 mg/kg	4,05 ± 0,38 p < 0,001	1,33 ± 0,09 p < 0,001	0,38 ± 0,02 p < 0,001

Note: p - reliability of differences between the indices in the intact and experimental groups

Thus, in the mucous layer of the large intestine of rats, Cefix increased urease activity by 73,2 %, Sumamed – by 46,9 %, Amoxiclav – by 32,1%, lincomycin – by 116,3% and anti-Helicobacter complex-by 130,1 %. These data indicate increased reproduction of pathogenic microbiota in the colon mucosa after antibiotic therapy. At the same time increased the activity of urease and serum. The most marked changes after the introduction of rats lincomycin (by 83,5 %) and anti-Helicobacter complex (79,7 %).

In the liver of rats, Sumamed and Amoxiclav did not cause a significant increase in urease activity ($p > 0,1$-$0,2$), whereas after the application of Cefix, Lincomycin, this index increased by 1,57 times ($p < 0,05$). The course of anti-Helicobacter therapy caused an increase in urease activity in the liver of rats by 1,81 times ($p < 0,001$).

Summarizing the results of table 2, it should be noted that urease activity is most significantly increased in the mucosa layer of the colon, serum and liver of rats after a course of injection of Lincomycin and H. pylori complex. Research O. I. Tsyryuk confirm the development of dysbiosis in the stomach of rats after long-term use of omeprazole, a component of anti-Helicobacter therapy [9]. Application Amoksiklav did not cause significant changes in urease activity in the studied tissues, and the introduction. Sumamed increased this index is minimal.

The results of the study of blood serum of experimental animals, given in table 3, characterize the state of hepatocytes (alanine aminotransferase and alkaline phosphatase activity), lipid metabolism (triglyceride content) and the degree of inflammation (elastase activity). Course administration of drugs 1 series, with the exception of Amoxiclav, led to a significant increase in the activity of ALT: after the use of Cefix by 31,4 %, Sumamed – by 27,1 %, lincomycin – by 64,3 %, anti- Helicobacter therapy – by 50,0 %. Amoxiclav caused a slight (18,6 %, $p > 0,2$) increase in the activity of this transaminase (tabl. 3).

Along with the increased activity of ALT in the blood serum of rats, there was a significant increase in other "hepatic" marker – the activity of alkaline phosphatase. The introduction of Cefix increased the activity of this enzyme by 80,1 %, Sumamed – by 27,9 %, Amoxiclav – by 12,9 %, lincomycin – by 49,8 %, anti-Helicobacter complex – by 59,3 % (table. 3).

The use of antibiotics in all cases led to disruption of lipid metabolism, as evidenced by the increase in triglyceride levels in the blood serum of animals. Thus, in the first series, this figure increased by an average of more than 70 % after the course of Cefix, Amoxiclav and Lincomycin. In the blood serum of rats treated with Sumamed, there was a less

pronounced increase in triglycerides by 38,9 %. Course of complex therapy of H. pylori increased the level of triglycerides in blood serum by 41,1 %.

Table 3

Biochemical parameters in the blood serum of rats after antibiotic use

Groups of rats	ALT activity, mc-kat/l	Activity of alkaline phosphatase, μ-kat / l	Triglyceride content, mmol/l	Activity of elastase, mc-kat/l
1th series				
Intact	0,70 ± 0,08	4,12 ± 0,13	0,90 ± 0,06	125,4 ± 3,3
Cefix, 20 mg/kg	0,92 ± 0,06 p < 0,05	7,42 ± 0,34 p < 0,001	1,54 ± 0,15 p < 0,002	95,9 ± 3,8 p < 0,01
Sumamed, 25 mg/kg	0,89 ± 0,03 p < 0,05	5,27 ± 0,22 p < 0,01	1,25 ± 0,14 p < 0,05	108,7 ± 6,4 p < 0,05
Amoxiclav, 40 mg/kg	0,83 ± 0,02 p > 0,2	4,65 ± 0,17 p < 0,05	1,60 ± 0,17 p < 0,002	102,7 ± 7,8 p < 0,02
Lincomycin, 60 mg/kg	1,15 ± 0,05 p < 0,001	6,17 ± 0,51 p < 0,002	1,59 ± 0,20 p < 0,01	101,4 ± 2,7 p < 0,01
2nd series				
Intact	0,38 ± 0,03	0,91 ±0,08	1,12 ± 0,14	140,5 ± 8,9
Omeprazole 1,3 mg/kg Amoxyl, 50 mg/kg Clarithromycin, 7,5 mg/kg	0,57 ± 0,04 p < 0,002	1,45 ± 0,12 p < 0,002	1,58 ± 0,18 p < 0,05	177,2 ± 5,7 p < 0,01

Note: p-reliability of differences between indicators in intact and experimental groups

The activity of leukocyte elastase, reflecting the degree of inflammation in the body, was significantly reduced after administration of antibiotics in 1th series. In contrast, the use of anti-Helicobacter complex caused an increase in the activity of elastase in the blood serum of rats by 24, 4 % (p < 0,01, tabl. 3).

Thus, this study demonstrates that the use of Lincomycin and anti-Helicobacter complex is most significantly disturb the functional performance of the liver and Amoxiclav and Sumamed have a minimal effect on these parameters (tabl. 3).

Figure 1 presents the calculated data on the degree of colon dysbiosis, increased urease activity in the liver and serum ALT activity. From the above data it is clear that the higher the degree of colon dysbiosis after taking different antibiotics, the higher the activity of ALT in the blood serum and the higher the activity of urease in the liver and conversely. The most aggressive drugs on the results of our study were Lincomycin and anti-Helicobacter complex, and most gentle – Amoxiclav and Sumamed.

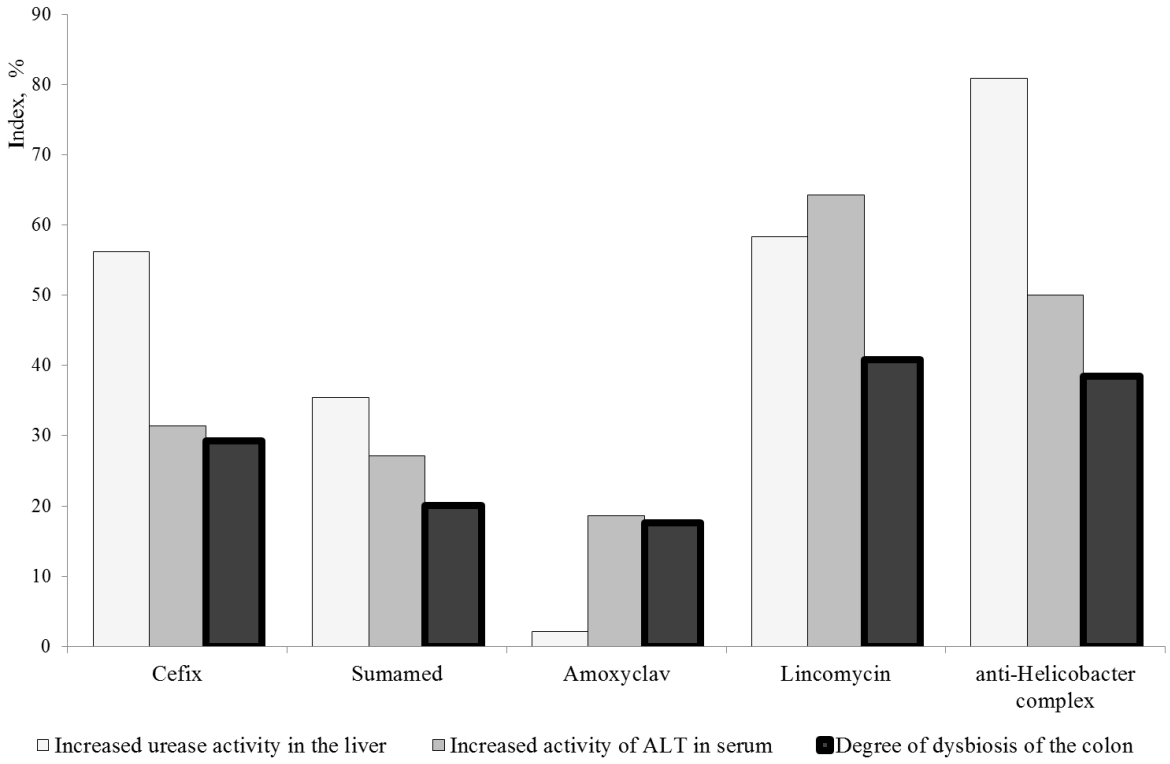

Figure 1. The effect of antibiotics on the degree of intestinal dysbiosis, increased urease activity in the liver and increased ALT activity in rat serum

Based on these results, it can be assumed that urease of pathogenic bacteria of the colon, getting into the liver with blood, has a toxic effect on the functional activity of hepatocytes. It is known that the development of dysbiosis and the increase in the absolute number of toxicogenic strains negatively affects all systems and organs [10] due to the increased concentration of microbial exo- and endotoxins [11, 12], which, in our opinion, can be attributed to urease.

Thus, the conducted researches allowed to make the assumption about hepatotoxic action of urease of microbiota of intestines after antibiotic therapy. It is important to emphasize that the rate of Amoksiklav or Sumamed causes minimal changes of intestinal dysbiosis and dysfunction of the hepatocytes.

Since there are no other drugs that can so powerfully and quickly cope with the infection, as antibiotics, the results dictate the need to find effective protective agents for the preservation of intestinal microbiocenosis during antibiotic therapy.

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