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LIPOPOLISACHARID DISRUPTS THE FUNCTION OF the LIVER IN DYSBIOSIS

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Abstract

The paper presents the results of a study of the effect of lincomycin and lipopolysaccharide on the degree of dysbiosis in the intestinal mucosa and indicators of the functional activity of the liver of laboratory rats.

It is established, that the introduction of lincomycin and lipopolysaccharide increases the degree of dysbiosis in the mucosa of the colon, increases the activity of alkaline phosphatase and elastase in the liver, and also increases the level of bilirubin, the activity of alkaline phosphatase and alanine aminotransferase in the blood serum of animals. Based on the data obtained, it is assumed that the cause of functional disorders of the liver is lipopolysaccharide, which produces opportunistic bacteria in dysbiosis.

Key words: dysbiosis, antibiotic, lipopolysaccharide, functional parameters of the liver.

The liver is exposed to numerical pathogenic factors: viruses, bacteria, parasites, and toxic substances of both exogenous and endogenous origin, a number of medicinal products (primarily antibiotics). All this leads to the development of hepatotoxicity pathology, which is manifested in the form of hepatosises, cholestasis, hepatitis, which leads to ultimate cirrhosis or hepatocellular cancer [1, 2, 3].

One of the most important hepatic functions is its ability to neutralize the toxic substances that are formed in the body (endotoxins) or come from the outside (exotoxins). This antitoxic function of the liver plays an important role in the transformation of drugs, the destruction of hormones and other biologically active substances [4].

In our previous work, the negative effect of various antibiotics on the functional parameters of the liver was shown, and also the assumption that in the development of dysbiosis after the administration of antibiotics a role played by a decrease in the activity of the antimicrobial enzyme lysozyme in the liver and the colon [5]. Currently, it is not clear what factors other than antibiotics themselves can disrupt the liver function in dysbiosis. In this regard, the purpose of our work was a comparative study of the influence of the antibiotic lincomycin and endotoxin lipopolysaccharide from Salmonella typhi on the functional characteristics of the liver of rats.

Materials and methods

The study was conducted on 24 male rats of the Vistar line at the age of 4 months and weighing 153 ± 12 g, which were divided into 3 groups of 8 animals: 1 - intact; 2 □ rats, which were administered lincomycin 50 mg/kg with drinking water for 5 days; 3 rats injected with a lipopolysaccharide from *Salmonella typhi* Pirogenal (Medgamal, Russia) at a dose of 10 mg/kg, administered intramuscularly daily for 5 days.

Euthanasia of rats was performed under the thiopental anesthesia (20 mg / kg) on day 8 after the end of the administration of drugs, blood was collected for serum, separated the area of the liver and colon. The colon was washed with distilled water and isolated mucous layer. Homogenates of the tissues were prepared at a rate of 50 mg / ml of a 0,05M Tris-HCl buffer pH 7,5. For biochemical studies, supernatant was used which was obtained after centrifugation at 2500 rpm for 30 minutes.

The urease [6] and lysozyme activity [7] were measured in the mucous layer of the colon, and their relative activity was based on the degree of dysbiosis [8]. In the liver of animals, the activity of elastase [9] and alkaline phosphatase [10] was determined. In serum, determination of the activity of alanine aminotransferase (ALT), alkaline phosphatase, and

bilirubin content was performed [10]. Statistical processing of the results was carried out using the parametric t-criterion of Student and the program "Statistica". [11].

Results of the research and discussion

The results of Table 1 indicate that the administration of lincomycin, as well as lipolysaccharide, leads to a significant increase in urease activity in the mucous layer of the large intestine by 3.4-4.2 times ($p < 0,001$). These data suggest a significant increase and reproduction in the mucous layer of the colon of conditionally pathogenic microbiota, which produces urease.

Table 1

Influence of lincomycin and lipopolysaccharide on the activity of urease and lysozyme in the mucosa layer of the large intestine of rats.

№	Groups	Urease activity, μ-kat/kg	Activity of lysozyme unit/kg	Degree of dysbiosis
1	Intact	0,72 ± 0,13	140 ± 16	1,00 ± 0,01
2	Lincomycin	2,47 ± 0,25 p < 0,001	57 ± 9 p < 0,001	8,37 ± 1,02 p < 0,001
3	Lipopolysaccharide	3,05 ± 0,45 p < 0,001	73 ± 10 p < 0,001	7,91 ± 0,94 p < 0,001

Note. p - the reliability of the differences from the values in the intact group

Urease is an aggressive hemotoxic factor for monocytes and neutrophils, reducing their functional activity and contributing to the proliferation of opportunistic bacteria. The final product of urease NH_3 is able to induce the production of superoxidanium and singlet oxygen radicals by neutrophils ("oxygen explosion").

In the mucous layer of the colon under the influence of pathogenic factors there was a significant decrease in the activity of the antimicrobial factor of lysozyme: after the introduction of lincomycin - in 2,4 times, and after lipopolysaccharide – in 1,9 times. The obtained results indicate a decrease in nonspecific antimicrobial protection of the colon mucous after the introduction of the substances under test. The logical consequence of the decrease in the activity of lysozyme is the reduction of the degree of antimicrobial protection, and hence the growth and reproduction of opportunistic microbiota.

The degree of dysbiosis, calculated on the relative activity of urease and lysozyme, in the mucous layer of the large intestine increases significantly after the administration of both drugs (Table 1). Thus, the introduction of lincomycin contributed to an increase in the degree of dysbiosis in the mucous layer of the large intestine in 8,37 times ($p < 0,001$), and the introduction of the lipopolysaccharide – in 7,91 ($p < 0,001$).

Thus, the data in Table 1 show that the course of antibiotic treatment of a wide spectrum of action or the introduction of endotoxin from Salmonella typhi negatively affects nonspecific antimicrobial protection, which leads to the reproduction of opportunistic microbiota and ultimately to the development of dysbiosis, which confirms a significant increase in degree dysbiosis in the mucous layer of the colon of rats.

In tabl. 2 show changes in the level of biochemical markers of inflammation in the liver tissue of rats given lincomycin or lipopolysaccharide. As can be seen from these data, both markers of inflammation are significantly increased by an average of 1,5-2,0 times, both after receiving lincomycin rats and under the influence of lipopolysaccharide.

Leukocyte elastase is considered a marker of chronic and acute inflammatory diseases, an indicator of secretory degranulation and activation of neutrophilic leukocytes, which indicates the presence of inflammation. An increase in the activity of alkaline phosphatase is the result of increased enzyme synthesis by cells located in the bile duct, usually in response to cholestasis, which may be intrathecal and extrahepatic origin. Therefore, a significant increase in the activity of elastase and alkaline phosphatase in the liver tissue is evidence of cholestasis and inflammation (Table 2).

Table 2

Influence of lincomycin and lipopolysaccharide on the activity of inflammatory markers in the liver of rats

№	Group	Activity elastase, μ-kat/kg	Activity of alkaline phosphatase, μ-kat/kg
1	Intact	266 ± 18	3,31 ± 0,11
2	Lincomycin	447 ± 30 $p < 0,001$	3,99 ± 0,29 $p < 0,05$
3	Lipopolysaccharide	463 ± 55 $p < 0,01$	3,84 ± 0,24 $p < 0,05$

Note. p - the reliability of the differences from the values in the intact group

In tabl. 3 results of the definiti layer on of "liver" markers of ALT, alkaline phosphatase and bilirubin in blood serum of rats, with experimental pathology. It is evident from these data that lincomycin and lipopolysaccharide contributed to an increase in the level of bilirubin by 46,4%, the activity of ALT - an average of 61,5%, and alkaline phosphatase - an average of 56,2%.

Increasing activity of ALT along with an increase in the level of bilirubin indicates damage to hepatocytes, and the activity of alkaline phosphatase - on manifestations of cholestasis.

Table 3

Influence of lincomycin and lipopolysaccharide on the level of liver serum markers in rats

№	Group	The content of bilirubin, μmol/l	Activity of AlAT, μ-kat/l	Activity of alkaline phosphatase, m-kat/l
1	Intact	2,52 ± 0,44	0,13 ± 0,02	0,89 ± 0,07
2	Lincomycin	3,68 ± 0,27 p < 0,05	0,22 ± 0,03 p < 0,05	1,46 ± 0,06 p < 0,05
3	Lipopolysaccharide	3,70 ± 0,41 p < 0,05	0,20 ± 0,04 p > 0,05	1,32 ± 0,06 p < 0,05

Note. p - the reliability of the differences from the values in the intact group

Thus, our studies convincingly prove that the administration of lincomycin or lipopolysaccharide causes, along with the development of intestinal dysbiosis, damage to hepatocytes, as evidenced by a significant increase in the level of biochemical "liver" markers (ALT, alkaline phosphatase and bilirubin) in serum of experimental animals. This is confirmed by an increase in the markers of inflammation in the liver tissue (the activity of elastase and alkaline phosphatase).

In our previous experiment, after antibiotic therapy, increasing urease activity and the degree of dysbiosis in the colon was due to a decrease in the synthesis of lysozyme by the liver [5]. Maybe, lincomycin and lipopolysaccharide damage hepatocytes and they can not fully synthesize lysozyme [12]. As a result, in the mucous layer of the colon, the conditionally pathogenic microbiota develops and grows predominantly. The proof of this is the increased activity of urease in the mucous layer of the colon, both after the introduction of lincomycin, and after the introduction of the lipopolysaccharide.

Conditionally pathogenic microbiota, namely gram-negative bacteria, produces excess amounts of the endotoxin (lipopolysaccharide), which enters the liver in the portal vein. Hepatocytes of the liver, partially destroyed by the antibiotic, are not capable of neutralizing, a large amount of endotoxin and, under its influence, initiates an additional destruction of hepatocytes. This is confirmed by other authors [13, 14]. Such a mechanism of development of pathology can be attributed to the "vicious circle". The established facts give a significant basis for the use of hepatoprotectors for the treatment of patients with intestinal dysbiosis, and as etiotropic factors of hepatobiliary pathology - to recommend the use of antidisbiotic drugs.

Conclusions

1. Introduction lincomycin or lipopolysaccharide cause in the mucous layer of the large intestine of rats sharp (8 times) increase the degree of dysbiosis.

2. In the liver of rats under the influence layer of lincomycin and lipopolysaccharide, the activity of alkaline phosphatase and elastase increases, indicating the development of inflammatory reaction in the liver tissue and manifestations of cholestasis.

3. Biochemical analysis of rat serum confirmed the development of hepatolysis after the administration of lincomycin or lipopolysaccharide: an increase in the level of total bilirubin, an increase in the activity of alkaline phosphatase and alanine aminotransferase.

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