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INFLUENCE OF DYSBIOTIC SYNDROME ON THE FORMATION OF EXPERIMENTAL NEPROPATHY

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Abstract

Kidneys are sensitive to damage by pathogenic factors of dysbiotic syndrome, among which lipopolysaccharide is the main one. However, the influence of the dysbiotic syndrome on the formation of nephropathy has not been studied enough.

The aim of the study is to determine the effect on the kidneys of rats of an experimental dysbiotic syndrome, which was modeled using the antibiotic lincomycin in combination with the administration of epinephrine.

Material and methods. Experimental dysbiotic syndrome (systemic dysbiosis) was reproduced in 21 Wistar rats (female, 11 months old, average weight 310±25 g) by administration of lincomycin with adrenaline hydrotartrate. The activity of urease and lysozyme was determined in blood serum and tissue homogenates and the degree of dysbiosis was calculated. The condition of the kidneys was assessed by the activity of elastase, urease, lysozyme, catalase, the content of malondialdehyde (MDA), creatinine, indicators of antioxidant protection.

Results. The level of urease increases in the liver of rats with experimental dysbiosis by 2.3 times, in the mucous membrane of the stomach - by two times, and in blood serum - by 2.3 times. The activity of lysozyme decreases in rats with dysbiosis: in the liver - by 42%, in

the stomach - by 36%, and in blood serum - by 32%. Urease activity in the kidneys increases by 76%, lysozyme activity decreases by 33%, which indicates a 2.6-fold increase in the degree of dysbiosis. As a result of dysbiosis in the kidneys, the level of biochemical markers of inflammation significantly increases: elastase by 79.5% and MDA by 18%, catalase activity significantly decreases (by 6%).

Conclusions. The combined administration of lincomycin and adrenaline causes the development of a dysbiotic syndrome in experimental animals. Under the condition of modeling the dysbiotic syndrome, an inflammatory-dystrophic process develops in the kidneys, and the level of antioxidant protection significantly decreases. Under conditions of experimental dysbiosis, pathological processes in the kidneys are manifested to a lesser extent than in other organs, possibly due to the high initial level of lysozyme in the tissue.

Key words. Dysbiosis; nephropathy; systemic inflammatory response syndrome; pro- and antioxidant system; pathogenesis; microbiota; kidney.

ВПЛИВ ДИСБІОТИЧНОГО СИНДРОМУ НА ФОРМУВАННЯ ЕКСПЕРИМЕНТАЛЬНОЇ НЕФРОПАТІЇ

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Резюме.

Нирки чутливі до ураження патогенними факторами дисбіотичного синдрому, серед яких основним є ліпополісахарид. Проте, вплив дисбіотичного синдрому на формування нефропатії досліджено недостатньо.

Мета дослідження – визначити вплив на стан нирок щурів експериментального дисбіотичного синдрому, який моделювали з використанням антибіотика лінкоміцину у поєднанні з уведенням адреналіну.

Матеріал і методи. Експериментальний дисбіотичний синдром (системний дисбіоз) відтворювали у 21 щура лінії Вістар (самиці, віком 11 місяців, середньою масою 310±25 г), адмініструванням лінкоміцину з адреналіном гідротартрату. У сироватці крові і в гомогенатах тканин визначали активність уреази, лізоциму і розраховували ступінь дисбіозу. Стан нирок оцінювали за активністю еластази, уреази,

лізоциму, каталази, вмісту малонового діальдегіду (МДА), креатиніну, показників антиоксидантного захисту.

Результати. Рівень уреази підвищується в печінці щурів з експериментальним дисбіозом у 2,3 раза, у слизовій оболонці шлунку – удвічі і в сироватці крові – у 2,3 раза. Активність лізоциму знижується у щурів з дисбіозом: у печінці – на 42 %, у шлунку – на 36 % і в сироватці крові – на 32 %. Активність уреази в нирках підвищується на 76 %, активність лізоциму знижується на 33 %, що свідчить про збільшення ступеня дисбіозу у 2,6 раза. У результаті дисбіозу у нирках суттєво зростає рівень біохімічних маркерів запалення: еластази на 79,5 % і МДА на 18 %, достовірно знижується активність каталази (на 6 %).

Висновки. Поєднане уведення лінкоміцину й адреналіну викликає розвиток дисбіотичного синдрому в експериментальних тварин. За умови моделювання дисбіотичного синдрому, в нирках розвивається запально-дистрофічний процес, суттєво знижується рівень антиоксидантного захисту. За умови експериментального дисбіозу, патологічні процеси в нирках проявляються дещо меншою мірою, ніж в інших органах, можливо, за рахунок високого вихідного рівня лізоциму в тканині.

Ключові слова. **Дисбіоз; нефропатія; синдром системної запальної відповіді; про- та антиоксидантна система; патогенез; мікробіота; нирки.**

Introduction

It is well known that the kidneys are very sensitive to damage by pathogenic factors of the dysbiotic syndrome [1], the main of which is lipopolysaccharide (LPS) [2]. It is known that the oral cavity is one of the most important sources of microbes and their toxins in the body, rivaling only the large intestine. However, if on the way of intestinal microbes and their toxins to the internal environment there is a specific organ - the liver, which performs its barrier antimicrobial function, then there are practically no obstacles for oral microbes and their toxins on the way to penetrate into the bloodstream. It is important to note that the complex of involved pathogenetic mechanisms is complex and not fully understood [3-5].

Entering the blood from the oral cavity, endotoxins (the so-called stomatogenic endotoxinemia) can cause the development of a number of somatic diseases, primarily heart and lung pathologies. There are also data on liver disorders that occur under conditions of stomatogenic endotoxinemia, and already then the possibilities of antimicrobial protection of hepatocytes are reduced, i.e. a vicious circle is closed [6-8].

The oral cavity contains a large number and variety of microorganisms, second only to the large intestine. Microbial pathogenic factors from the oral cavity easily penetrate into the circulatory system and, bypassing the barrier function of the liver, have a negative effect on all organs, in particular on the kidneys [9-11].

It is also known that the severity of the dysbiotic syndrome (for example, an increase in the level of bacteremia) significantly increases against the background of stress, which increases the degree of dysbiosis [12, 13].

The purpose of the study

The aim of this study was to determine the effect on the kidneys of rats of an experimental dysbiotic syndrome, which was simulated using the antibiotic lincomycin in combination with the administration of adrenaline.

Material and methods

Experimental dysbiotic syndrome (EDS), or systemic dysbiosis, was reproduced in rats that were given lincomycin with drinking water at a dose of 60 mg/kg during the first five days of the experiment. We used lincomycin in ampoules of 2 ml of 30% solution, produced by PJSC "Pharmaceutical Firm "Darnytsia" (Ukraine), adrenaline hydrotartrate in ampoules of 1 ml of 0.18% solution, produced by LLC "Pharmaceutical Company "Zdorovya" (Ukraine).

To increase the degree of dysbiosis in experimental animals, starting from the 7th day of the experiment, oral applications of a gel containing adrenaline (0.18 mg/ml) in a dose of 1 ml/kg were made for 3 days, i.e. simulating one of the stress mechanisms.

The study used 21 Wistar rats (females, 11 months old, average weight 310±25 g), divided into three equal groups: 1st – control, 2nd – received adrenaline, 3rd – dysbiosis on the background of adrenaline stress The study was carried out in strict compliance with ethical national and international regulations and recommendations for the humane treatment of experimental animals.

On the 10th day, rats were euthanized under thiopental anesthesia (20 mg/kg) by total bleeding from the heart. Blood serum was obtained, kidneys, liver and gastric mucosa were removed. The activity of urease and lysozyme was determined in blood serum and tissue homogenates and the degree of dysbiosis was calculated. The activity of elastase, catalase, and malondialdehyde content were also determined in kidney homogenates, and the antioxidant-prooxidant index of API was calculated based on the ratio of catalase activity and malondialdehyde content.

The condition of the kidneys was evaluated by the following biochemical indicators: elastase activity and malondialdehyde (MDA) content (indicators of the inflammatory process), catalase activity and the antioxidant-prooxidant index API (antioxidant protection indicators), urease activity (indicator of bacterial insemination), lysozyme activity (indicator of non-specific immunity) and the degree of dysbiosis and the content of creatinine in blood serum as a marker of the level of glomerular filtration.

Statistical processing by the method of variational statistics using the MS Excel 365 program. With a value of $p<0.05$, the difference in results between groups was considered probable.

Research results

The first stage was the determination of markers of dysbiosis: activity of urease, lysozyme, the degree of dysbiosis in the liver, gastric mucosa and blood serum, which allows for a comparative analysis of the obtained data (Table 1).

Table 1 - Activity of urease, lysozyme and degree of dysbiosis in various organs of rats with experimental dysbiotic syndrome (x±Sx)

№	organs and groups	urease	lysozyme	degree of dysbiosis
Liver				
1	Control (n=7)	0,26±0,09	179,4±13,28	1,00±0,15
2	EDS (n=7)	0,61±0,10 p=0,024	104,3±12,61 p<0,01	4,05±0,38 p<0,001
Mucous membrane of the stomach				
1	Control (n=7)	0,21±0,05	149,5±8,33	1,00±0,18
2	EDS (n=7)	0,42±0,08 p=0,046	95,28±15,61 p=0,0098	3,12±0,29 p=0,0015
Blood serum				
1	Control (n=7)	0,66±0,21	107,4±8,41	1,00±0,17
2	EDS (n=7)	1,51±0,28 p=0,032	73,65±5,18 p=0,014	3,87±0,42 p=0,0014

Note. p – the degree of reliability, compared to the control.

From the data in the table. 1, it can be seen that the level of urease increases in the liver of rats with experimental dysbiosis by 2.3 times, in the mucous membrane of the stomach - twice, and in blood serum - by 2.3 times, which indicates the possibility of a significant increase in bacterial insemination of these tissues. As a result, the activity of lysozyme in these tissues, on the contrary, decreases in rats with dysbiosis: in the liver - by 42%, in the stomach - by 36%, and in blood serum - by 32%, which indicates a significant decrease in the level of non-specific immunity, which can be associated with the consequences of dysbiosis.

It should be noted that the kidneys have a very high lysozyme activity, which is ten times higher than its level in other tissues, which indicates the importance of antibacterial protection (Fig. 1).

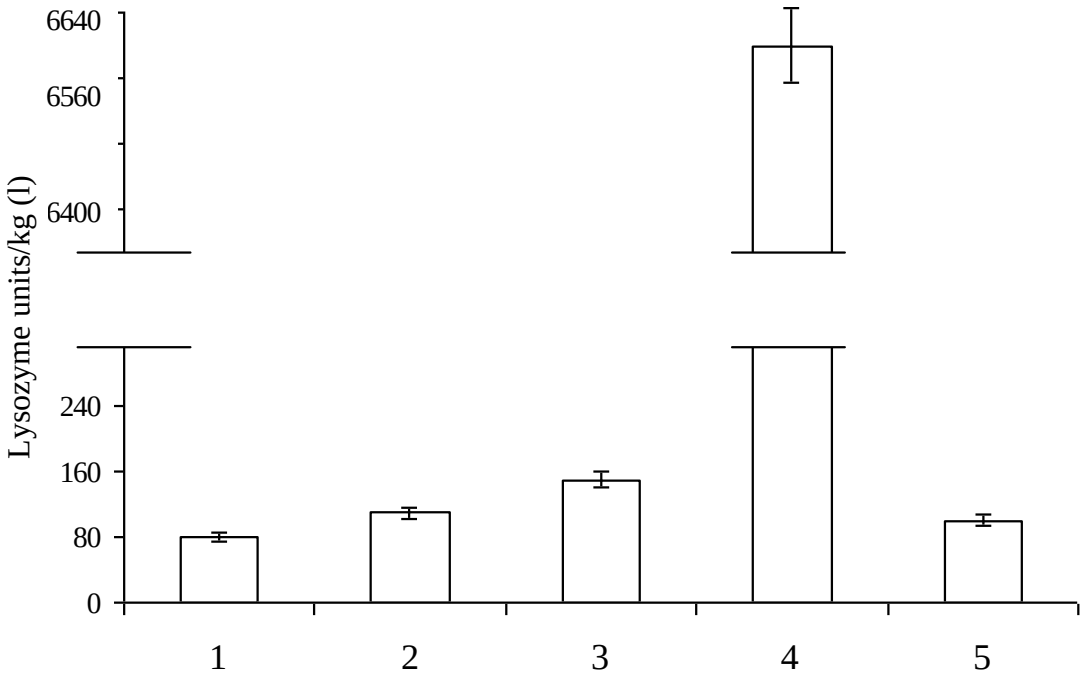

Fig. 1. Comparison of lysozyme activity in different tissues of rats:
1 – gums; 2 – stomach; 3 – liver; 4 – kidneys; 5 - blood serum

In contrast to lysozyme, urease activity in intact kidneys is very low (10 times less than in the oral cavity), which indicates the absence of microflora in the kidneys of healthy animals (Fig. 2).

As a result of administration of lincomycin and adrenaline, the degree of dysbiosis in the liver of rats increases 4 times, in the stomach - 3.1 times, and in blood serum - 3.9 times.

The obtained data indicate the development of generalized dysbiosis, i.e. dysbiotic syndrome.

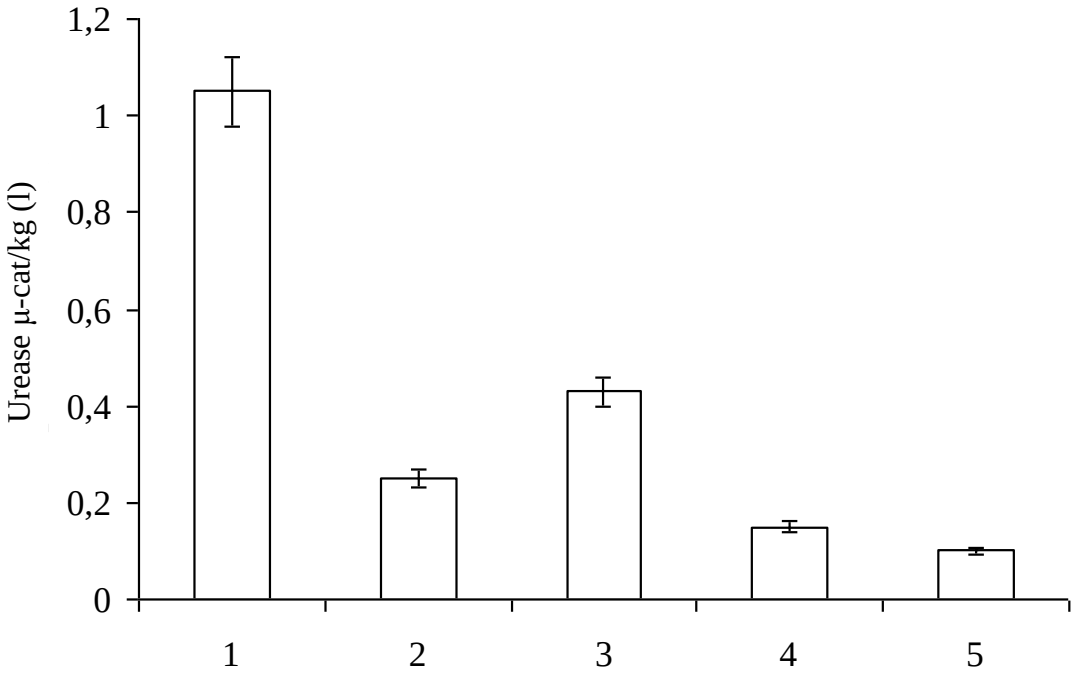

Fig. 2. Comparison of urease activity in different tissues of rats:
1 – gums; 2 – stomach; 3 – liver; 4 – kidneys; 5 - blood serum

Table 2 shows the results of determining the activity of urease, lysozyme and the degree of dysbiosis in the kidney homogenate of rats with experimental dysbiosis. From these data, it can be seen that in rats, after the administration of lincomycin and adrenaline, the activity of urease in the kidneys increases by 76%, while the activity of lysozyme decreases by 33%, which indicates a 2.6-fold increase in the degree of dysbiosis.

At the same time, the dysbiotic process develops in the kidneys to a lesser extent than in other organs, possibly due to the very high activity of the antimicrobial enzyme lysozyme, the level of which in the kidneys exceeds that in all other organs and tissues of the body.

However, as a result of dysbiosis (Table 3), the level of biochemical markers of inflammation in the kidneys significantly increases: elastase by 79.5% and MDA by 18 %.

Table 2 - The activity of urease, lysozyme and the degree of dysbiosis in the kidneys of rats with experimental dysbiotic syndrome (x±Sx)

№	Groups	urease, μ-cat/kg	lysozyme, units/kg	degree of dysbiosis, units
1	Control (n=7)	0,17±0,09	6621±475,5	1,00±0,20
2	Adrenalin (n=7)	0,22±0,12 p>0,5	9846±629,3 p=0,0062	0,87±0,11 p>0,5
3	Experimental dysbiotic syndrome (EDS) (n=7)	0,30±0,10 p>0,05	8050±386,2 p=0,038	1,44±0,02 p<0,05

Note. p – the degree of reliability, comparing with the control.

Table 3 - The level of inflammatory markers in the kidneys of rats with experimental dysbiotic syndrome

№	Groups	Elastase, μ-cat/kg	MDA, mmol/kg
1	Control (n=7)	440,6±70,35	35,52±1,24
2	Adrenalin (n=7)	460,2±27,54 p>0,05	37,46±1,32 p>0,05
3	Experimental dysbiotic syndrome (EDS) (n=7)	790,3±130,4 p=0,036	41,93±1,97 p=0,017

Note. p – the degree of reliability, comparing with the control.

At the same time, kidneys with dysbiosis are also characterized by high activity of the proteolytic enzyme elastase (Fig. 3), the source of which is neutrophils [14, 15].

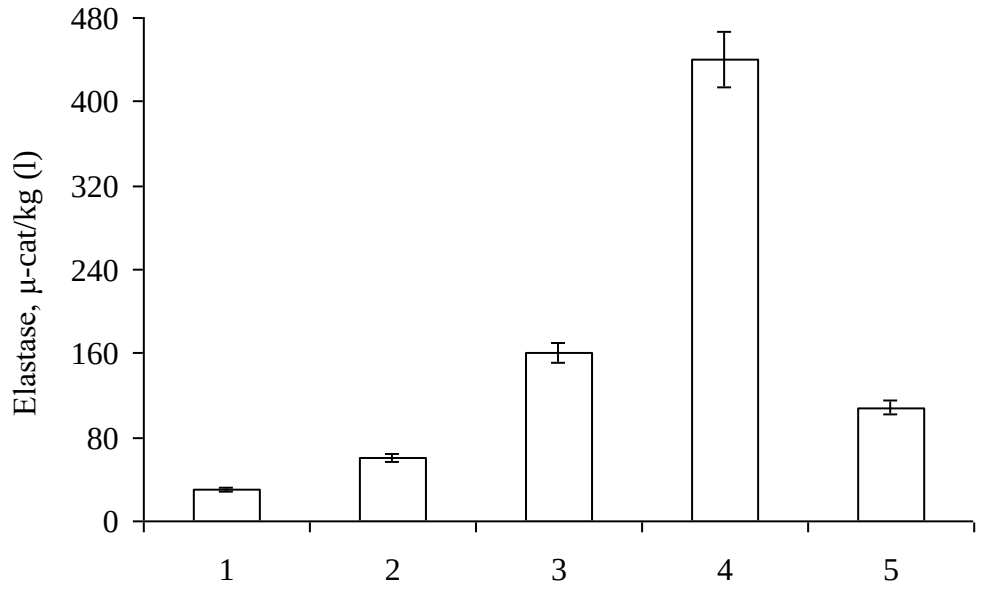

Fig. 3. Comparison of elastase activity in rat tissues with dysbiosis

1 – gums; 2 – stomach; 3 – liver; 4 – kidneys; 5 - blood serum

That is, the occurrence of dysbiosis in the kidneys due to dysbacteriosis causes the development of inflammation, in which neutrophils participate.

In order to find out the role of biochemical mechanisms in the development of inflammation in the kidneys, indicators characterizing the activity of lipid peroxidation processes were studied (Table. 4).

Table 4 - Catalase activity and API index in the kidneys of rats with experimental dysbiotic syndrome (x±Sx)

№№	Groups	Catalase, mcat/kg	API, unit
1	Control (n=7)	6,30±0,05	1,78±0,08
3	Experimental dysbiotic syndrome (EDS) (n=7)	5,93±0,10 p=0,013	1,41±0,09 p=0,0095

Note. p – the degree of reliability, comparing with the control.

Listed in the table. 4 results of determination of catalase activity and antioxidant-prooxidant index in the kidneys show that catalase activity (by 6%) and API index (by 21%) are significantly reduced in rats with dysbiosis.

Thus, in the kidneys under experimental dysbiosis, the processes of activation of peroxidation are involved in the development of inflammation, which also leads to a decrease in antioxidant protection.

Conclusions

The combined administration of lincomycin and adrenaline causes the development of a dysbiotic syndrome in experimental animals.

Under the condition of modeling the dysbiotic syndrome, an inflammatory-dystrophic process develops in the kidneys, and the level of antioxidant protection significantly decreases.

Under conditions of experimental dysbiosis, pathological processes in the kidneys are manifested to a somewhat lesser extent than in other organs, possibly due to the high initial level of lysozyme in the tissue.

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