Levitsky A. P., Vasjuk V. L., Levchenko E. M. Influence of antidysbiotic drugs on the liver of rats with experimental non-alcoholic steatohepatitis. Journal of Education, Health and Sport. 2017;7(11):402-408. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.1461661
http://ojs.ukw.edu.pl/index.php/johs/article/view/6179

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 1223 (26.01.2017).
1223 Journal of Education, Health and Sport eISSN 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 06.11.2017. Revised: 20.11.2017. Accepted: 30.11.2017.

INFLUENCE OF ANTIDYSBIOTIC DRUGS ON THE LIVER OF RATS WITH EXPERIMENTAL NON-ALCOHOLIC STEATOHEPATITIS

A. P. Levitsky, V. L. Vasjuk*, E. M. Levchenko**

State Establishment "The Institute of Stomatology and Maxillo-facial Surgery NAMS of Ukraine"
***Bukovinian State University**
****Municipal Institution "Odessa Regional Hospital"**

High-fat diets combined with the introduction of lincomycin causes the development of steatohepatitis. Introduction antidysbiotic drugs (pro- and prebiotics) has a therapeutic effect.

Key words: steatohepatitis, dysbiosis, antidisbiotic agents, probiotics, prebiotics.

Non-alcoholic steatohepatitis (NASH) occurs in 2-4% of the population [14], which in Ukraine is 1-2 million people. NASH develops as a result of liver obesity, currently defined as non-alcoholic fatty liver disease (NGBP), which occurs in 10-40% of the population [14]. NAFS develops in 4 stages: steatosis, steatohepatitis, fibrosis and cirrhosis. The causes of NAFW are increased fat intake [21] and the presence of dysbiosis [20], which cause the development of insulin resistance. The latter syndrome underlies the pathogenesis of diabetes mellitus type 2, metabolic syndrome, obesity and atherosclerosis [4]. Based on the data on the important role of dysbiosis in the pathogenesis of NAFB, we proposed to use antidisbiotic

agents (ADS) for the prevention of steatohepatitis, in particular, such drugs as pro-and prebiotics [9].

Recently, it was proposed to use the complex preparation Kvertutulin containing bioflavonoid quercetin, the prebiotic inulin and calcium citrate as ADS [11]. To enhance the immunostimulatory properties of Quertulin, we added Imudon [16] and called this drug "Quertulidone" [12]. The purpose of this study was to determine the therapeutic efficacy in experimental steatohepatitis combined use of oral applications of Quertulidon and multiprobiotics "Simbiter".

Material and research methods. The experiments were carried out on 24 8-month-old male Wistar white rats weighing 200 ± 15 g divided into 3 groups: 1 — control, 2 and 3 — experimental model of stetohepatitis (ESG), group 3 received oral applications of gels "Quertulidon "And" Simbiter "0.3 ml per rat daily for 21 days.

Experimental steatohepatitis was caused by keeping rats on a high-fat diet (+25% to the standard feed of a mixture of palm oil and heat-treated soy flour in a 1: 1 ratio) and reproducing intestinal dysbiosis (lincomycin with drinking water at the rate of 70 mg / kg body weight during the first 5 days) [17]. Phytogel "Quertulidon" (quertulin - 3%, "Immudon" - 8 mg, mint extract - 10%, sodium benzoate - 2%, menthol - 0.1%, carboxymethylcellulose sodium salt - 4%, distilled water - up to 100%) . RC U 20.4-13903778-032/8: 2015 and TU U 20.4-13903778-032: 2012, produced by LLC Biokhimtekh (Odessa). Probiotic phytogel "Simbiter" (acidophilic, concentrated) produced by O. D. Prolisok "with. V. Vilshanka Vasilkovsky district of Kiev region.) - 10%, mint extract - 10%, sodium benzoate - 2%, carboxymethylcellulose sodium salt - 4%, distilled water - up to 100%). RC U 20.4-13903778-032/2: 2012 and TU U 20.4-13903778-032: 2012. produced by LLC "Biokhimtekh", Odessa. Symbiotic symbiotics also includes lactobacilli and lactococci 6-1010 CFU / g, bifidobacteria 1-1010 CFU / g, and acetic acid bacteria 1-106 CFU / g [1].

The killing of animals was carried out on the 22nd day of the experiment under thiopental anesthesia (20 mg / kg) by total bleeding from the heart. Blood serum and liver tissue were stored until –30 ° C. In the liver homogenate (50 mg / ml 0.05 M Tris-HCl buffer pH 7.5), the level of inflammation markers was determined [7]: the content of malondialdehyde (MDA) by reaction with thiobarbituric acid [15] and elastase activity by hydrolysis of the synthetic substrate [13].

Alkaline phosphatase (alkaline phosphatase) activity [10], urease activity (microbial contamination marker) [2], lysozyme activity by the bacteriolytic method [6] were determined, and the degree of dysbiosis according to A. P. Levitsky [8]. In addition, total

cholesterol (TC) [19] and triglycerides (TG) [5] were determined in the liver. The level of liver markers was determined in blood serum: alanine aminotransferase activity (ALT) [3] and alkaline phosphatase activity (AP) [10], OX and TG levels. Statistical processing of the obtained data was carried out in accordance with the recommendations [18]. To calculate the significance of differences used the t-student criterion.

Results and its discussion. In rats with ESH in the liver, an increase in cholesterol level was noted (by 25.6%), and in serum - a significant increase in its level (by 32.6%). The use of ADS somewhat reduces the level of cholesterol in the liver and in the serum, however, in both cases P> 0.05 (Fig. 1).

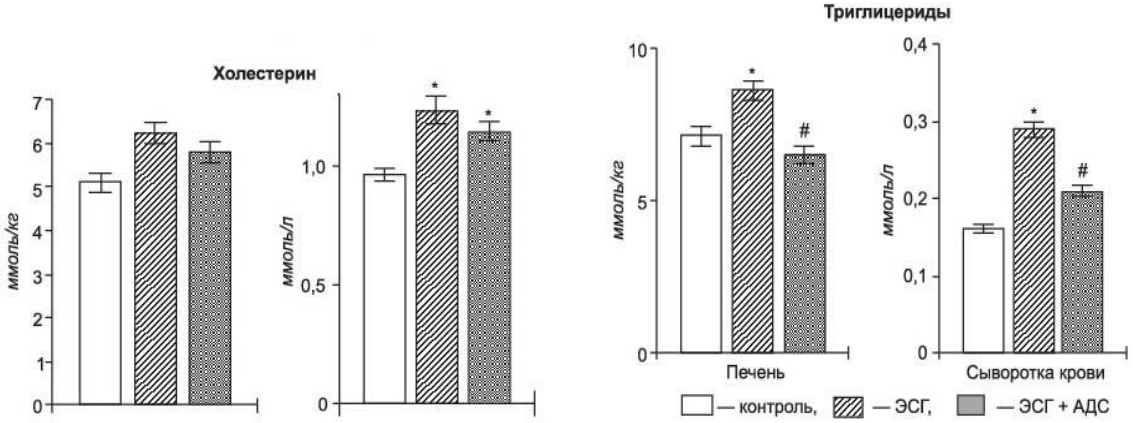

Fig. 1. Effect of BPA on cholesterol and triglycerides in liver tissue and serum in rats with ESH. * - P <0.05 compared with the control, # - P <0.05 compared with ESG.

At the same time, the level of TG in the liver significantly increased by 16.3% and in serum by 83.0% (see Fig. 1). Oral applications of anti-disbiotic drugs (Quertulidon and Simbiter gels) significantly reduce their level in the liver and in blood serum. Fig. 1. Effect of BPA on cholesterol and triglycerides in liver tissue and serum in rats with ESH. * - P <0.05 compared with the control, # - P <0.05 compared with ESG. In rats with ESH, inflammation markers significantly increased - the level of MDA in the liver (by 55.3%), in serum (by 15.2%) and the activity of elastase in the liver (by 56.0%), in blood serum (by 53 , 1%) (Fig. 2). The use of ADS significantly reduces the level of MDA in the liver (by 59.2%) and in serum (by 9.4%). However, after oral administration of ADS, there was only a tendency to a decrease in the elastase activity both in the liver and in serum (see Fig. 2).

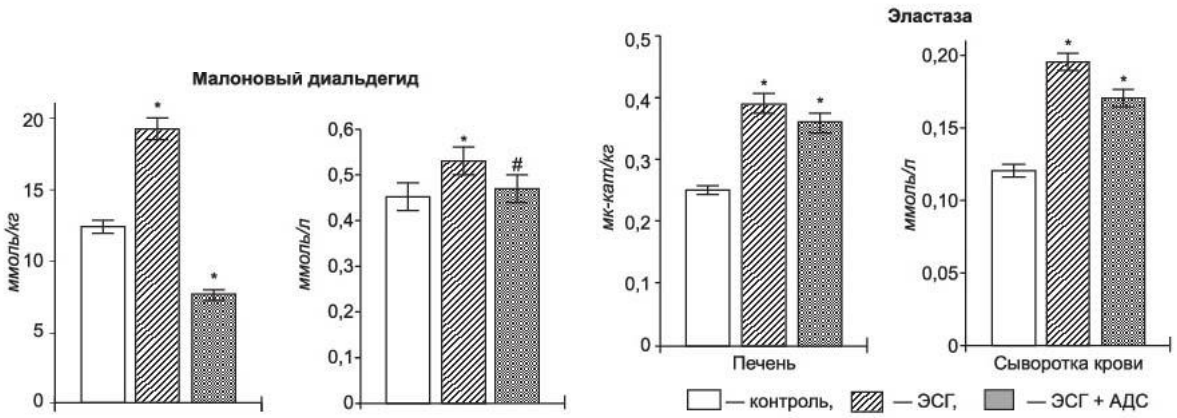

Fig. 2. Effect of ADF on the level of malondialdehyde and elastase activity in liver tissue and serum in rats with ESG. * - P <0.05 compared with control.

In the serum of rats with ESH, the activity of hepatic markers of ALT and alkaline phosphatase increased statistically significantly by 41.5% and 73.7%, respectively. Oral applications of ADS only slightly reduce their level (Fig. 3).

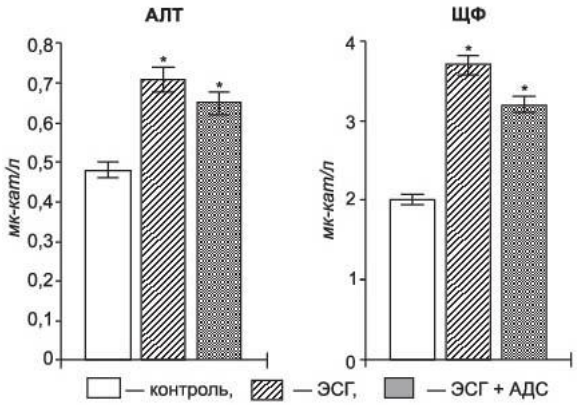

Fig. 3. Effect of BPA on serum ALT and alkaline phosphatase activity in rats with ESG. * - P <0.05 compared with control.

In rats with ESG, urease activity increases by 50%, which indicates an increase in microbial contamination (Fig. 4).

Lysozyme activity, in contrast, is reduced by 60.4%, indicating a significant decrease in the level of nonspecific immunity. After oral administration of ADS, there was only a tendency to a decrease in the activity of urease and to an increase in the activity of lysozyme. The degree of dysbiosis calculated by these indicators in the liver of rats with ESH increases

by 3.8 times. Oral applications of ADS significantly reduce the degree of dysbiosis, but do not return it to normal.

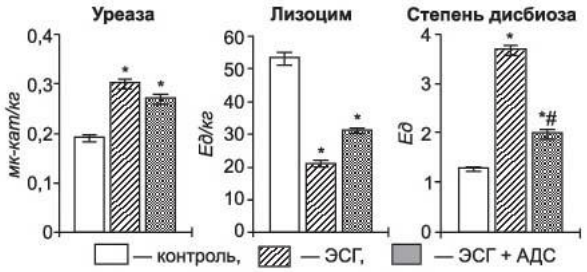

Fig. 4. Effect of ADS on urease activity, serum lysozyme level and degree of dysbiosis in ESH rats. * - P <0.05 compared with the control, # - P <0.05 compared with ESG.

Conclusions 1. High-fat diet and antibiotic administration cause the development of non-alcoholic steatohepatitis (NASH).

2. NASH is accompanied by the development of dysbiosis in the liver.

3. Anti-disbiotic agents (pro-and prebiotics, immunostimulants) have a therapeutic and prophylactic effect in NASH.

References

1. Voronkova A. V., Smaglyuk L. V., Levitskiy A. P. Lechebno-profilakticheskoye deystviye simbiotika «Simbiter» pri eksperimental'nom stomatite // Ukraїns'kiy stomatologíchniy al'manakh. — 2013. — № 1. — S. 5-7.

2. Gavrikova L. M., Segen' I. T. Ureaznaya aktivnost' rotovoy zhidkosti u bol'nykh s ostroy odontogennoy infektsiyey chelyustno-litsevoy oblasti // Stomatologiya. — 1996. — Spets. vypusk. — S. 49-50.

3. Goryachkovskiy A. M. Klinicheskaya biokhimiya v laboratornoy diagnostike // [3-ye izd.]. — Odessa: Ekologiya, 2005. — 616 s.

4. Yegorova Ye. G. Insulinorezistentnost' — osnova metabolicheskogo sindroma // Eksperimental'naya i klinicheskaya gastroenterologiya. — 2007. — № 1. — S. 8-13.

5. Instruktsiya do naboru reaktyviv dlya vyznachennya tryhlitserydiv u syrovattsi i plazmi krovi enzymatychnym kolorymetrychnym metodom / TU U 24.4-24607793-020-2003.

6. Levitskiy A. P. Lizotsim vmesto antibiotikov. — Odessa: KP OGT, 2005. — 74 s.

7. Levitskiy A. P., Den'ga O. V., Makarenko O. A. i dr. Biokhimicheskiye markery vospaleniya tkaney rotovoy polosti: metod. rekomendatsii. — Odessa: KP OGT, 2010. —16 s..

8. Levyts′kyy A. P., Dyen′ha O. V., Selivans′ka I. O. ta in. Sposib otsinky stupenya dysbiozu (dysbakteriozu) orhaniv i tkanyn. Patent na korysnu model′ № 43140. MPK 2009 G01N 33/48. № u 2008 15092 vid 26.12.2008. Opubl. 10.08.2009. Byul.№ 15.

9. Levitskiy A. P., Levchenko Ye. M., Vasyuk V. L. Gepatoprotektornoye deystviye antidisbioticheskikh preparatov pri eksperimental'nom metabolicheskom sindrome // Zhurn. NAMN Ukrainy. — 2014. — 20, № 4. — S.478-482.

10. Levitskiy A. P., Makarenko O. A., Den'ga O. V. i dr. Eksperimental'nyye metody issledovaniya stimulyatorov osteogeneza: metodicheskiye rekomendatsii. — K.: GFTS, 2005. — 50 s.

11. Levitskiy A. P., Makarenko O. A., Selivanskaya I. A. i dr. Kvertulin: vitamin R, prebiotik, gepatoprotektor. — Odessa: KP OGT, 2012. — 20 s.

12. Levitskiy A. P., Selivanskaya I. A., Ivanov V. S. i dr. Lechebnoye deystviye preparata “Kvertulin” na sostoyaniye tkaney polosti rta krys pri antikhelikobakternoy terapii // Byulleten' KHIV chteniy im. V. V. Podvysotskogo. — Odessa: Feniks, 2015. — S.113-114.

13. Levitskiy A. P., Stefanov A. V. Metody opredeleniya aktivnosti elastazy i yeye ingibitorov: metodicheskiye rekomendatsii. — K.: GFTS, 2002. — 15 s.

14. Mekhtiyev S. N., Grinkevich V. B., Kravchuk YU. A. i dr. Nealkogol'naya zhirovaya bolezn' pecheni: klinika, diagnostika i lecheniye // Lechashchiy vrach. — 2008. — № 2. — S. 29- 37.

15. Stal'naya I. D., Garishvili T. G. Metod opredeleniya malonovogo dial'degida s pomoshch'yu tiobarbiturovoy kisloty // V kn.: Sovremennyye metody v biokhimii. — M.: Meditsina, 1977. — S.66-68.

16. Sysoyev N. P., Kaladze N. N. Otsenka effektivnosti mestnoy immunokorregiruyushchey terapii preparatom imudon v kompleksnom lechenii zabolevaniy parodonta u detey s revmatoidnym artritom // Sovremennaya stomatologiya. — 2004. — № 1. — S.60-62.

17. Tkachuk V. V., Velychko V. I., Levchenko O. M. ta in. Vplyv dysbiozu na stan pechinky ta lipidnoho obminu shchuriv, yaki otrymuvaly vysokozhyrovyy ratsion // Odes′kyy med. zhurn. — 2014. — № 2(142). — S. 27-31.

18. Trukhacheva N. V. Matematicheskaya statistika v medikobiologicheskikh issledovaniyakh s primeneniyem paketa Statistica. — M.: GEOTAR-Media, 2012. — 379 s.

19. Kholesterin. Fermentativnyy-fotometricheskiy metod s kholesterin-oksidazoy (peroksidazoy) / RT MD 11-15796482-001. — 2003.

20. Cani P. D., Biliboni R., Knauf C. et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice // Diabetes. — 2008. — 57, № 6. — P. 1470-1481.

21. Uetake Y., Ikeda H., Irie R. et al. High-salt in addition to high-fat diet may enhance inflammation and fibrosis in liver steatosis induced by oxidative stress and dyslipidemia in mice // Lipids in Health and Disease. — 2015. — 14, № 6. — P. 1-8.