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Research Article

AN EFFECT OF THE MULTIPROBIOTIC "SYMBITER ACIDOPHILIC" CONCENTRATED ON THE LEVEL OF EXPRESSION OF mRNA GENE *Ptgs2* AND *Tgfb1* IN RATS LIVER WITH MONOSODIUM GLUTAMATE-INDUCED STEATOHEPATOSIS

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A B S T R A C T

The studies were conducted on the experiment with 30 white male rats. Newborn infant rats were divided into 3 groups with 10 rats in each group. The first group performed as a control value. The second group rats in the early neonatal period were injected with monosodium glutamate (MSG) at a dose of 4 mg/g. These rats were accessed to have visceral adiposity and steatohepatosis. The third group rats (after neonatal injection of MSG) periodically were injected multiprobiotic "Symbiter acidophilic concentrated" (0,14 ml/kg orally) starting from the first month after the birth. In the rats liver of 4 month old we determined the level of expression of mRNA gene *Ptgs2* and *Tgfb1*.
In comparison with animals of control group, in the rats liver after neonatal injection of MSG the level of expression of mRNA gene *Ptgs2* which involved in the inflammatory conditions was increased in 8,2 times and the level of expression of mRNA gene *Tgfb1* which involved in extension of fibrosis in the liver tissues was increased in 1,7 times. The rats with steatohepatosis (after neonatal injection of MSG) which were injected with "Symbiter acidophilic concentrated", the levels of mRNA gene *Ptgs2* and *Tgfb1* expression returned to control values.

INTRODUCTION

For now, the statistics of obesity in the world is considered to be a global epidemic (Rubinstein and Low, 2017). Steatohepatosis or non-alcoholic fatty liver disease draws special attention among the complications of metabolic disorders, and that is considered to be one of the most spread forms of major metabolic disorders and that is a non-specific, integral and multifactorial liver injury in the presence of obesity and metabolic syndrome.

It is commonly known that the first stage of liver damage, followed by inflammatory conditions in the cells, is conditioned by oxidative and nitrosative stress. Damaged hepatocytes and biliary cells secrete proinflammatory cytokines and factors that activate Kupffer cells and stimulate T-lymphocytes activity. In chronic liver damage, activation of star liver cells with inflammatory and fibrogenic properties is observed (Friedman *et al.* 2007). Currently, there exists a range of literature on the features of expression of dozens of genes in the liver on different stages of inflammatory conditions, fibrosis, cirrhosis, etc. (Lee *et al.* 2018; Marcolongo *et al.* 2009; Viswanadha and Loor, 2012; Feillet-Coudray *et al.* 2019; Ulmasov *et al.* 2018) including the *Ptgs2 gene* (Henkel *et al.* 2018; Cha *et al.* 2018) and *Tgfb1 gene* (*Lancha et al.* 2014).

Factors that lead to obesity and as the result to steatohepatosis include excessive consumption of monosodium glutamate (MSG) which is one of the world's most widely used food additives and is found especially in fast food (Ninomiya,1998; Hernández Bautista *et al.* 2019). He K. *et al.* (2011) hypothesized that MSG is positively associated with weight gain, which influences energy balance through the disruption of the hypothalamic signaling cascade of leptin action.

No references were found in the search terms "obesity and monosodium glutamate and Ptgs2 gene" or "obesity and monosodium glutamate and Tgfb1 gene" in the PubMed. In the search terms "obesity and liver fibrosis and monosodium glutamate and Ptgs2 gene" or "obesity and liver fibrosis and monosodium glutamate and Tgfb1 gene" in the PubMed we found the literature about connection between liver fibrosis and *Ptgs2 gene* and also *Tgfb1 gene*.

Most of medications for treatment of obesity have severe side effects. This is the reason for their withdrawal from production. Only Orlistat could be taken by patients during long time.

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However, little attention is paid to the search of means for prophylaxis of obesity. In current scientific literature there are a lot of studies that confirm beneficial effects of probiotics on human organism. The question about probiotics impact on fat metabolism and obesity is being actively debated in the scientific literature. The gut microbiota has been recently proposed to be an environmental factor involved in the control of body weight and energy homeostasis (Backhed *et al.* 2004, 2005; Ley, 2017; Turnbaugh *et al*. 2006, 2008).

Formerly we have shown that multiprobiotic "Symbiter acidophilic" concentrated therapy from childhood prevents the development of nonalcoholic fatty liver disease in adult monosodium glutamate-induced obese rats (Kondro *et al.* 2014).

So, the paper is aimed to study the effect of a multi-probiotic "Symbiter acidophilic concentrated" on the level of mRNA and *Ptgs2* and *Tgfb1* genes expression in the rats` liver with MSG-evoked steatohepatosis.

MATHERIALS AND METHODS

Experiments conducted by following general principles of bioethics in accordance with international recommendations of European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and approved by First National Congress for Bioethics (September 2001).

Experiment design and experimental groups.

The study involved 30 white non-linear rats males, born by several males with a difference of 1-3 days. Newborn infant rats were randomly divided into 3 groups with 10 rats in each group. The first group performed to have control: they were injected subcutaneously the physiological salt solution at a dose of 8 mql/g.

Newborn infant rats of the second and third groups were injected subcutaneously MSG at a dose of 4 mg/g in the solved physiological salt solution at 2^{nd}, 4^{th}, 6^{th}, 8^{th} and 10^{th} days of life (Sanabria, 2002). The volume of the injected solution of (MSG) comprised 8 mql/g of infant rats weight. Starting from the first month after rats were born and next three months the rats of the first group (control) and second (after neonatal injection of MSG) were injected orally 0,5 ml of water at a periodical basis. The third group rats (after neonatal injection of MSG) starting from the first month after birth and next three months were injected orally multiprobiotic "Symbiter acidophilic" concentrated at a periodical basis (produced by research and production company O.D. Prolisok, Ukraine) at a dose of 0,14 ml/kg in the solved 0,5ml of water for injections (a conclusion by the state sanitary-epidemiological examination №5.03.03.-04/37/92, 08.09.2003). Periodical injections of water or multi-probiotic were conducted according to the next scheme: 2 weeks of injection, 2 weeks of break and maintenance of the standard feeding.

Multiprobiotic "Symbiter acidophilic" concentrated contains at least $1x10^{11}$ of CFU/g of living of symbiosis cells of 14 blocks of probiotic bacteria which belong to the class of Bifidobacterium, Lactobacillus, Lactococcus, Propionibacterium i Acetobacter and are placed in mutual interconnections (Yankovsky *et al*. 2009, 2010).

The microorganisms of the specimen "Symbiter" are resistant to the stomach acid, digestive enzymes, lysozyme, bile acids which enable them to get through all the sections of the intestinal tract.

At the age of 4 months, the rats of all groups were sacrificed by the method of cervical dislocation, their liver was separated and the studies were conducted on the level of genes expression by the methods mentioned below.

Real-time RT-PCR.

RNA was obtained by the method of Chomczynski (1987). The synthesis of cDNA and real-time quantitative polymerase chain reaction (Real-time PCR) using the commercial kit "Thermo Scientific Verso SYBR Green 1-Step qRT-PCR ROX Mix" ("Thermo Scientific", Lithuania) with 0, 4 μmol/l of each primer was carried out according to the recommended temperature conditions: cDNA 50°C synthesis - 30 min; initiating denaturation 95 °C - 15 min; next 40 cycles: denaturation of DNA 95 °C - 15 s; hybridization of primers 50 - 53 ºC - 35 s; completion of the chain 72 °C - 30 s; elongation of amplitudes 72 °C - 5 min.

There were used the following primers for Tgfb1 reactions: direct- CTTCAGCTCCACAGAGAAGAACTG and conversed CACGATCATGTTGGACAACTGCTCC; for Ptgs2 direct – TGCTGTTCCAACCCATGTCA and conversed - TGTCAGAAACTCAGGCGTAGT; for Actb (β-actin gene used for internal control of reaction due to constitutive expression) – direct – TGGGACGATATGGAGAAGAT and conversed – ATTGCCGATAGTGATGACCT.

The reproduction of the amplification results was checked in the parallel experiments by replication of PCR on the examples of RNA of all rats with each prime at least three times. After each cycle of amplification the fluorescence of the SYBR Green I colorant was retrieved and after the reaction was completed, a melting line chart was constructed to control the formation of the primer dimers and the specifics of the reaction. The primary quantity of mRNA was calculated by the comparative CT method «ΔΔCT Method», the efficacy of PCR reactions was the same (Ex =(10-1/slope)-1), slope < 0,1. The related quantity of mRNA genes was normalized to mRNA Actb.

Statistial Methods

Statistic processing of studies results: the obtained data was tested on normal distribution by the Shapiro–Wilk test with the usage of software GraphPad Prism 5.04 - 7 (GraphPad Software Inc., USA). The following calculation was performed by one-way ANOVA with Tukey's range test. The obtained results were presented in the form of average arithmetical sign ±, the squared deviation (variance) - SD. The results were considered to be meaningful if $p \leq 0,05$.

THE RESULTS AND DISCUSSION

In the result of the studies conducted, it was determined that the level of expression of mRNA gene *Ptgs2* in the group of controlling animals equaled to 1,000±0,155 in relation to β-actin (Fig. 1). In the group of animals with MSG-evoked steatohepatosis this level increased in 8,2 times (p=0,001) in comparison with animals of control value (Fig. 1). The results are conditioned with the numerous researches which demonstrate the increase of expression of pro-inflammatory

cytokine of mRNA genes in inflammatory processes of the liver, including the *Ptgs2* genes (Henkel *et al.* 2018; Cha *et al.* 2018).

The level of expression of mRNA gene *Ptgs2* of the rats with MSG-evoked steatohepatosis, which were periodically injected multi-probiotic "Symbiter acidophilic" concentrated, was 9,1 times ($p<0,001$) lower than the animals with steatohepatosis and was not different statistically from the ones of control values.

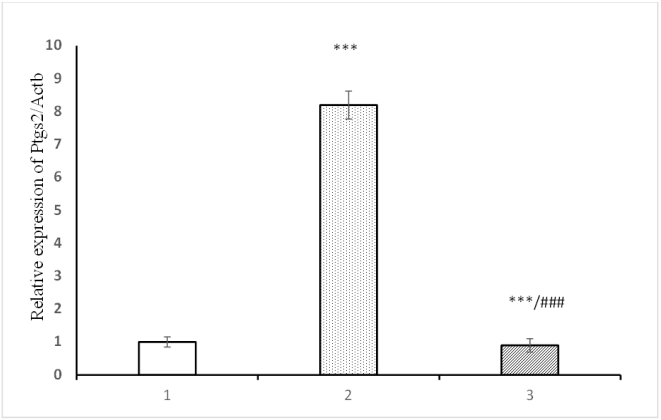

Fig 1 The level of expression of mRNA gene *Ptgs2* in the rats` liver with MSG-evoked steatohepatosis and the level of those which were injected multiprobiotic "Symbiter acidophilic" concentrated M±SD (n=10):

1. control value, 2- steatohepatosis , 3 - steatohepatosis + multi-probiotic,
2. *** - $p<0,001$ in comparison with intact animals (group of control);

– $p<0,001$ in relation to the animals with steatohepatosis.

It has been established that one of the elements of pathogenesis of hepatic steatosis of the liver is dysfunction of the β-oxidation of fatty acids and activation of lipid peroxidation (Buyever and Mayevskaya, 2003), which leads to the increased formation of free radicals (active forms of the oxygen, etc) of oxidative stress and resulted in cytotoxicity (Berezenko *et al.* 2014). As a result, hepatocytes become damaged which along with other cells of the liver produce inflammatory cytokines including prostaglandins E2.

Currently, the anti-inflammatory effect of probiotic specimens does not generate doubts. The study shows a drastic increase in the concentration of the eicosanoids in the stomach lining and blood serum of rats in the conditions of a lasting hypoacid state. Simultaneous injection of omeprazole and multiprobiotic "Symbiter acidophilic" concentrated leads to the proximity of eicosanoids to control values (Senin *et al*. 2010). The idea of one of the mechanisms of the determined phenomenon by the authors is a known feature of probiotic bacteria range including *Lactobacillus acidophilus* and *Bifidobacterium longum*, which are contained in the multiprobiotic.

Multi-probiotic «Symbiter acidophilic concentrated» is able to slow down a lipid peroxidation (Lin and Yen, 1999).

Expression of mRNA of another gene *Tgfb1* in the liver of rats with steatohepatosis has also been drastically changed. In the control values, it was equal to 1,000±0,127 in relation to β-actin and the rats with steatohepatosis had an expression of mRNA gene Tgfb increased to 1,7 times ($p<0,001$) and it was equal to 1,700±0,237 (Fig. 2). Detected increase of mRNA gene *Tgfb* expression can be related to the formation of fibrosis in the liver tissue as it was determined that one of the elements of mechanism of hepatocytes and epithelial cells transformation of bile duct is the increase of matrix synthesizing fibroblasts quantity in the liver with steatohepatosis due to the influence of the numerous mediators which facilitate increase of fibroblasts quantity including TGF-β1 and active forms of oxygen (Dooley *et al.* 2010; Liu and Gaston Pravia, 2010). Indeed, TGF-β1 is considered to be the main component of hepatocyte growing system, the formation of the fibrosis and apoptosis control (Dooley *et al*. 2010).

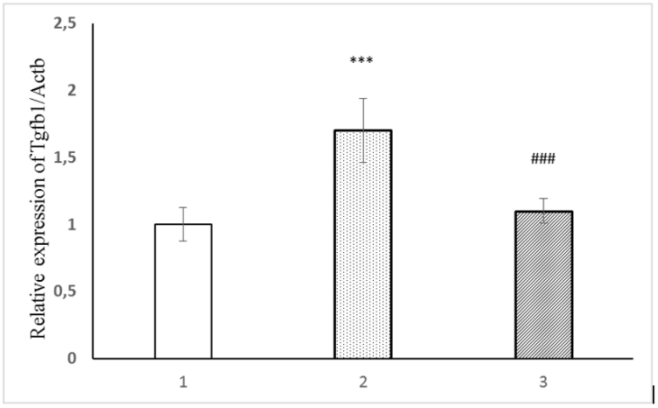

Fig 2 The level of expression of mRNA gene *Tgfb1* in the rats` liver with MSG-evoked steatohepatosis and the level of those which were injected multiprobiotic "Symbiter acidophilic" concentrated M±SD (n=10):

1-control value, 2- steatohepatosis, 3 - steatohepatosis + multiprobiotic,
*** - $p<0,001$ in comparison with the intact animals (group of control);
– $p<0,001$ in relation to the animals with MSG-induced steatohepatosis.

After injection of MSG in the early neonatal period and multi-probiotic "Symbiter acidophilic" concentrated on periodical basis, expression decreased by 1,55 times ($p<0,001$) in relation to the rats with steatohepatosis and did not have the statistical difference from the control value (Fig. 2) that is conditioned with previously obtained data on preventive influence of multiprobiotic "Symbiter acidophilic" concentrated on the formation of steatohepatosis of mature rats in the conditions of MSG injection in prenatal period (Savchenyuk *et al.* 2014).

Since the increase of *Ptgs2* gene expression which leads to the facilitation of pro-thyroid TGF-β1 (Wilgus *et al.* 2004), the decrease of expression level of mRNA gene Ptgs2 in the rats` liver with the effect of multiprobiotic "Symbiter acidophilic" concentrated with the injection of MSG in the early prenatal period allows to logically explain the decrease of expression level of mRNA gene Tgfb1 with regard to these conditions.

CONCLUSIONS

In the rats liver after neonatal injection of MSG the level of expression of mRNA gene Ptgs2 which involved in the inflammatory conditions was increased in 8,2 times ($p=0,001$) in comparison with animals of control group. The level of expression of mRNA gene Tgfb1 which involved in extension of fibrosis in the liver tissues was increased in 1,7 times ($p<0,001$) in comparison with animals of control group. The rats with steatohepatosis (after neonatal injection of MSG) which were injected with "Symbiter acidophilic" concentrated, had the levels of mRNA gene and Ptgs2 and Tgfb1 expression returned to control values. This gives the perspectives for the wide usage of multiprobiotic "Symbiter acidophilic" concentrated for preventive aims and treatment of steatohepatosis of different genesis.

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