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Expression of *Cckbr*, *Gast*, *Reg1*α, *Tgfb1* genes in rat pancreas upon long-term hypoacidity and with administration of multiprobiotic «Symbiter® acidophilic» concentrated

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Aim. *Determination of the Cckbr, Gast, Reg1*α *and Tgfb1 genes expression in rat pancreas upon long-term hypoacidity and with administration of multiprobiotic «Symbiter® acidophilic» concentrated.* ***Methods***. *Experiments were carried out on white non-strain mail rats. Hypoacidic state was modeled through intraperitoneal injection of omeprazole during 28 days. Level of genes expression was determined by semi-quantitative RT-PCR.* ***Results***. *The elevation of levels of Cckbr, Reg1*α *and Tgfb1 mRNAs, as well as the appearance of Gast gene expression in rat pancreas upon hypoacidic conditions were shown. The levels of Cckbr and Tgfb1 mRNAs with administration of multiprobiotic «Symbiter® acidophilic» concentrated under the same conditions were similar to the control, while the expression of Gast gene was not detected; at the same time, the level of Reg1*α *mRNA was higher than that in animals with hypoacidity.* ***Conclusions***. *Long-term hypoacidity is accompanied by changes in the expression of Cckbr, Gast, Reg1*α *and Tgfb1 genes in rat pancreas, while upon administration of multiprobiotic «Symbiter® acidophilic» concentrated the pattern of expression for most of the studied genes is similar to the control.*

Keywords: hypoacidity, pancreas, gene expression, multiprobiotics.

Introduction. Acid-related disorders are the most prevalent among gastroenterological diseases at present. In recent decades, proton-pump inhibitors (PPI) of the gastric parietal cells, such as omeprazole, remain the most effective therapeutic agents for this group of disorders [1].

It is proved for now that upon long-term use of PPIs the state of hypoacidity develops, which is accompanied by hypergastrinemia [2, 3]. It was shown in clinical trials that hypergastrinemia of any etiology may lead to the development of gastric atrophy and metaplasia, as well as sporadic tumors in other regions of gastrointestinal tract (GIT) and associated organs [2, 4, 5]. Furthermore, there is an evidence of an increased risk of acute pancreatitis development upon long-term use of PPIs [6].

According to scientific literature, the increased expression of *Reg1*α gene encoding eponymous protein is associated with regeneration of pancreatic islet cells and diabetogenesis upon the damage of gland [7, 8]. Moreover, it is shown that co-expression of *Cckbr* gene (codes gastrin/cholecystokinin receptor type B) and *Gast* gene (codes hormone gastrin) is common in human pancreatic adenocarcinoma [9, 10]. *Tgfb1* gene encoding isoform 1 of the transforming growth factor β (TGF-β1) is expressed in pancreatic cells upon normal conditions, but it is proved that its increased expression is associated with carcinogenesis and acute pancreatitis [11].

The development of dysbiosis is one of the key consequences of long-term hypoacidity. Colonization of GIT by opportunistic microbiota appears to be the stable sources of endogenous infection and additionally promotes gastric carcinogenesis [3, 5]. It is proved in clini-

cal trials that probiotics are able not only to cure dysbiotic states, but also to reduce the damage ratio of GIT immediately [12, 13].

Multiprobiotics of «Symbiter®» group (hereinafter referred to as Symbiter) are characterized by complexity, a wide array of bioactivity and composition maximally close to the natural microbial populations of human and animals [13].

Analysis of scientific literature showed a lack of data on the pattern of above mentioned genes expression in pancreas upon experimental or natural hypoacidity. Data on the effect of probiotics on gene expression in pancreas upon these conditions are also absent.

The aim of current investigation was to determine the expression of *Cckbr*, *Gast*, *Reg1*α and *Tgfb1* genes in rat pancreas upon long-term injection of omeprazole and with administration of Symbiter.

Materials and methods. The International recommendations on performance of medical and biological investigations with the use of animals according to European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes were followed. Experiments were carried out on white non-strain male rats with initial weight around 180–200 g.

All animals were divided into four groups. Rats injected with 0.2 ml of physiological solution abdominally and 0.5 ml of water for injections orally were used as a control (first group). Hypoacidity (second group) was modeled by everyday intraperitoneal injection of omeprazole (14 mg/kg) during 28 days [14]. The third experimental group simultaneously with omeprazole obtained Symbiter (manufactured by LLC «O. D. Prolisok», Ukraine) orally (0.14 ml/kg). Animals of the fourth group were treated with the same dose of Symbiter during 28 days. The number of animals in each experimental group was 8.

RNA was isolated following Chomczynski and Sacchi [15]; cDNA was synthesized in 20 μl of reaction mix containing 2 μg of RNA, 1 mM dNTP, 200 U of reverse transcriptase RevertAid M-MLV, corresponding buffer, 20 U of ribonuclease inhibitor, 20 pmol of reverse primer. Synthesis was carried out in the following conditions: 70 °C – 5 min, further 37 °C – 5 min, 42 °C – 1 h. Polymerase chain reaction was performed in 30 μl of reaction mix containing 10 μl of cDNA, PCR buffer, 200 μM of each dNTP, 30 pmol (1.0 μM) of each primer, 2.5 mM of $MgCl_2$ and 1.5 U of Taq DNA polymerase. PCR amplifications consisted of the initial denaturing step of 94 °C for 4 min, followed by 35 cycles (for β-actin – 30 cycles) of 94 °C for 45 s, appropriate annealing temperature for suitable time: *Cckbr* (184 b. p., 59 °C – 45 s), *Gast* (274 b. p., 52 °C – 40 s), *Reg1*α (608 b. p., 48 °C – 45 s), *Tgfb1* (298 b. p., 52 °C – 45 s) and β-actin (521 b. p., 49 °C – 40 s) (gene used as the internal control of reaction due to its constitutive expression); the final extension step at 72 °C for 1 min 15 s (for *Cckbr*, *Reg1*α and *Tgfb1*) or 1 min (for β-actin and *Gast*). Further fill-in of PCR products was performed upon 72 °C for 5 min.

The following primers were used in reactions: for *Cckbr* – forward – GCAAGCACGAGTATGGCAAA and reverse – TAGCACGGACCAGGTTTGTT; for *Gast* – forward – GCCCAGCCTCTCATCATC and reverse – GGGGACAGGGCTGAAGTG; for *Reg1*α – forward – AGCCTGCAGAGATTGTTGAC and reverse – CCATAGGGCAGTGAGGCAAG; for *Tgfb1* – forward – CTTCAGCTCCACAGAGAAGAACTGC and reverse – CACGATCATGTTGGACAACTGCTCC; for β-actin – forward – TGGGACGATATGGAGAAGAT and reverse – ATTGCCGATAGTGATGACCT. Separation of PCR products was performed electrophoretically in 1.6 % agarose gel with 0.5 × TBE buffer following Sambrook et al. [16]. For semi-quantitative analysis of amplicons expression based on densitometry the ImageJ 1.45s program was used. The indices of mRNA expression were calculated for each sample following Konturek et al. [17].

Statistical processing of experimental data was performed with analysis of variance [18]. Probability of difference between the control and test measurements was assessed with Student's *t*-test. The difference between compared data was treated as probable if $p < 0.05$. All calculations and graph plotting were carried out in «OriginLab Origin 8.6» and «Microsoft Excel 2003» programs.

Results and discussion. It was established that the mRNA level of *Cckbr* gene in the control was 0.578 ± ± 0.054 in relation to β-actin (Figure, *A*). In animals treated only with omeprazole for 28 days this parameter was 1.7 times higher in comparison with the control, while upon simultaneous administration of multiprobiotic Symbiter the level of *Cckbr* mRNA was two times lower than in the animals injected with omepra-

zole. In the animals treated only with Symbiter this parameter was 0.437 ± 0.041.

On the one hand, the indicated high level of *Cckbr* mRNA may be caused by the intensification of this gene expression only in pancreatic endocrine cells, but on the other hand, it may also be explained by the gain of expression in cells of exocrine pancreas [19]. This idea is supported by the literature data, according to which the expression of *Cckbr* gene in normal pancreas is shown for endocrine cells of α and δ subtypes (in both human and rat) [9, 20, 21]. Moreover, there are also the data on low *Cckbr* gene expression in pancreatic acinar cells in normal conditions [20, 21], while no corresponding mRNA is detected in duct cells.

In recent years, some data on the association between enormous expression of *Cckbr* gene and a number of pancreatic pathologies including acute pancreatitis were obtained [9, 20]. Furthermore, it was shown the relation between the *Cckbr* expression in acinar cells and carcinoma of this cellular type and pancreatic ducts adenocarcinoma [22]. Thus, the overexpression of *Cckbr* gene in pancreatic acinar cells is considered to be associated with carcinogenesis at the moment [20]. Unfortunately, the conditions of our experiment do not allow us to separate the role of specific cells in the established increase of gastrin receptor gene expression.

mRNA of *Gast* gene was not detected in rat pancreas of the control group (Figure, *B*). At the same time, upon long-term hypoacidity the expression of this gene was observed, since its mRNA was revealed in the samples of rat pancreases. The level of *Gast* gene mRNA in these conditions was 0.568 ± 0.067. All the while, expression of gastrin was observed neither in the third group (omeprazole + Symbiter), nor in the fourth (Symbiter).

Gastrin is expressed exclusively in enteroendocrine G-cells of gastric antrum mucosa and in proximal part of duodenum [21, 23]. According to the literature, «adventive» expression of gastrin may be associated with the development of gastrinoma; it is a common peculiarity of Zollinger-Ellison syndrome [23]. Moreover, it has been recently shown that in human pancreatic adenocarcinoma the co-expression of gastrin and CCK_BR proteins is observed [10, 20].

The gastrin mRNA was found only in pancreatic samples of animals treated with omeprazole during 28 days (Figure, *B*). At the same time, an increased level of the *Cckbr* mRNA was established upon these conditions (Figure, *A*), thus suggesting the co-expression of the corresponding genes. However, an increase of the *Cckbr* gene mRNA level may be limited only to endocrine cells, without any involvement of exocrine part of the pancreas [9, 20], so there is no sufficient basis for a suggestion about ductal adenocarcinoma existence upon long-term gastric hypoacidity. Further investigations are needed for the clarification of this aspect.

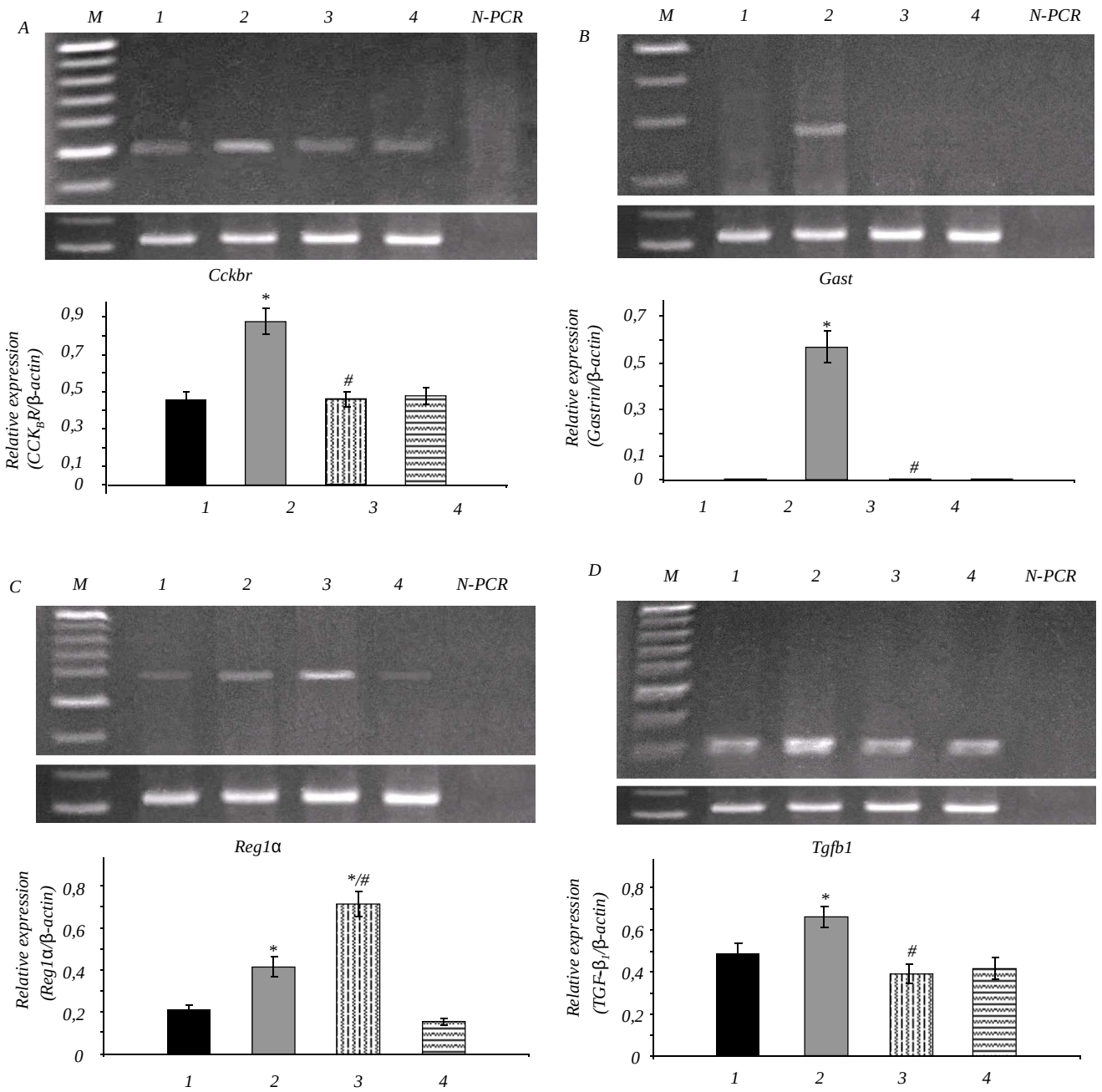
The investigation of *Reg1*α gene expression pattern showed that in the control the mRNA level was 0.211 ± ± 0.045 (Figure, *C*). In animals treated only with omeprazole for 28 days, the level of *Reg1*α mRNA was 0.414 ± 0.047, which is 2 times higher than the control values. Upon simultaneous administration of multiprobiotic Symbiter this parameter was 1.7 times higher than in animals of the second group. In animals treated only with Symbiter, similarly to the control, the level of *Reg1*α gene mRNA was 0.154 ± 0,012.

The *Reg1*α gene encodes a regenerative protein, which provides the formation of endocrine islands and regeneration of pancreatic tissue upon pathological conditions, and is also involved in the differentiation of pancreatic cells upon regeneration [7, 21]. This protein is constitutively expressed in pancreatic acinar cells, but not in islet or duct cells [8].

The increased level of *Reg1*α mRNA upon 28-day injection of omeprazole (Figure, *C*) may be connected with the regeneration of pancreas upon its damage and formation of new endocrine cells [7, 8, 21]. A higher level of mRNA upon simultaneous administration of omeprazole and Symbiter (in comparison with animals of the second group) indicates the intensification of regenerative processes in pancreas, which can promote more rapid regeneration of damaged tissues (primarily – endocrine cells).

The level of TGF-β1 mRNA was 0.485 ± 0.054 in the control (Figure, *D*). Upon long-term hypoacidity its level was 1.4 times higher in comparison with the control. At the same time, upon simultaneous administration of Symbiter the level of TGF-β1 mRNA was 1.7 times lower than in animals of the second group. In the fourth group this parameter was lower than in the control and amounted to 0.417 ± 0.051.

On the one hand, TGF-β1 is a potent oncosuppressor in normal cells and, on the other hand, is an oncopro-



Level of *Cckbr* (*A*), gastrin (*B*), *Reg1*α (*C*) and transforming growth factor β (*D*) mRNA in rat pancreas upon long-term hypoacidity and with administration of multiprobiotic «Symbiter® acidophilic» concentrated: *M* – molecular mass marker; *1* – control; *2* – omeprazole; *3* – omeprazole + + Symbiter; *4* – Symbiter; *N-PCR* – negative PCR control; *p < 0.05 in relation with control; #p < 0.05 in comparison with animals treated with omeprazole

moter in malignant cells [11]. Any disturbances in the expression of above mentioned protein may increase the risk of pancreatic carcinogenesis [11, 24]. The increase in TGF-β1 mRNA level in rat pancreas upon long-term hypoacidity was shown in our experiment. So, another assumption can be made about the existence of carcinogenesis in rat pancreas upon long-term administration of omeprazole.

Analysis of the recent scientific literature and the results of our experiments allow us to point out several possible mechanisms of the long-term hypoacidity effects on genes expression in the cells of rat pancreas.

Omeprazole can directly affect pancreatic cells. However, up to date there are no clear data about the possibility of such action. In particular, it was shown that omeprazole inhibits proliferation and modulates autophagy of several cell lines of pancreatic cancer [25]. However, these data are not appropriate for physiological conditions of a whole organism.

Another mechanism is the indirect effect of hypoacidity. As a consequence of low acid secretion in stomach, the qualitative and quantitative composition of GIT microbial population is disturbed, i. e. the pathological state of dysbiosis develops leading to formation of an endogenous infection source, including close quarters of the pancreas [26, 27]. There are rare assumptions in the literature about possible colonization of pancreatic ducts by dysbiotic microbiota, that during its livelihoods produce a number of bioactive substances, and among them N-nitroso compounds, the carcinogenicity of which is proved at the moment [28].

The effect of hypergastrinemia is of particular interest. The increase of serum gastrin is a compensatory response to the suppression of gastric acid secretion, since gastrin is a physiological stimulator of this process. According to the literature, in normal conditions the receptor for gastrin (CCK_BR) is expressed predominantly in endocrine pancreatic cells – in both human and rat [20].

The excess of gastrin in blood upon hypoacidity may form a substantial burden on pancreatic endocrine cells, thus changing the pattern of signal molecules secretion from these cells [29]. Aside from that, increased concentrations of gastrin can affect the peripheral blood lymphocytes, inducing the secretion of IFN-γ and IL-2, and also changing the activity of some intracellular enzymes, that may modulate the activity of these immune cells and their effects on the target cells [30].

Probably, the constellation of above mentioned factors exists, thus forming the image obtained in our experiment. It cannot also be excluded that a role of each factor varies depending on individual peculiarities of the organism, functional state of immune system, the whole term of hypoacidic state, etc.

Among probable mechanisms of Symbiter's action on gene expression in rat pancreas, firstly, it should be pointed out its ability to liquidate dysbiosis and bacterial colonization of GIT – it was observed in a number of investigations [13]. As a consequence, the burden of pathogenic microbiota is removed from GIT and associated organs.

Furthermore, Lutgendorff et al. [31] showed that multicomponent probiotics are able to increase *de novo* synthesis of the main low-molecular cellular antioxidant – reduced glutathione, and thus to raise its content in both GIT and pancreas. It is proved for now that an oxidative stress plays crucial role at the beginning stage of acute pancreatitis [32]. On the experimental model of acute pancreatitis Lutgendorff et al. indicated that the preliminary treatment with probiotics can ameliorate the rate of oxidative stress, inflammatory processes and damage of the pancreas [31].

The reduction of gastrin level in the blood upon administration of Symbiter has recently been observed [33]. Such effect may be associated with the decrease in pro-inflammatory cytokines IL-1β and IFN-γ levels in blood upon administration of probiotic, since it was shown that above mentioned cytokines promote the hypergastrinemia development through stimulation of gastrin gene expression in G-cells [34]. Based on these data, it may be suggested that observed effects of Symbiter are linked not only with normalization of GIT microbiota, but also with restriction of hypergastrinemia effects.

However, the final acceptance or rejection of this suggestion requires further investigations, which will allow us to explicitly distinguish the consequences of hypergastrinemia and bacterial colonization of GIT.

In summary, final elucidation of molecular mechaisms underlying the changes in expression of the *Cckbr*, *Gast*, *Reg1α* and *Tgfb1* genes in rat pancreas upon long-term hypoacidity and with administration of multiprobiotic Symbiter requires further more specialized and selective experiments.

Conclusions. Thus, we have shown that long-term experimental hypoacidity is accompanied by changes in the expression of *Cckbr*, *Gast*, *Reg1α* and *Tgfb1* genes in rat pancreas, while upon administration of multiprobiotic «Symbiter® acidophilic» the expression pattern of most of these genes is similar to the control. Based on these data, it can be assumed that there is some potential risk of pancreatic carcinogenesis upon long-term use of omeprazole (and probably other PPIs).

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Експресія генів *Cckbr, Gast, Reg1*α і *Tgfb1* у підшлунковій залозі щурів за умов тривалої гіпоацидності та при введенні мультипробіотика «Симбітер® ацидофільний» концентрований

Резюме

Мета. *Дослідити експресію генів Cckbr, Gast, Reg1*α *і Tgfb1 у підшлунковій залозі щурів за умов тривалої гіпоацидності та при введенні мультипробіотика «Симбітер® ацидофільний» концентрований.* ***Методи***. *Досліди проведено на білих нелінійних щурах-самцях. Гіпоацидний стан моделювали інтраперитонеальним введенням омепразолу протягом 28 діб. Рівень експресії генів визначали напівкількісним аналізом за допомогою ЗТ-ПЛР.* ***Результати***. *Показано зростання рівня мРНК Cckbr, Reg1*α *і Tgfb1, а також появу експресії гена Gast у підшлунковій залозі щурів за гіпоацидних умов. При введенні мультипробіотика «Симбітер® ацидофільний» концентрований за тих самих умов вміст мРНК Cckbr та Tgfb1 виявився на рівні контрольних значень, експресії гена Gast не зафіксовано, а рівень мРНК Reg1*α *перевищував цей показник тварин з гіпоацидним станом.* ***Висновки***. *Стан тривалої гіпоацидності супроводжується зміною експресії генів Cckbr, Gast, Reg1*α *і Tgfb1 у підшлунковій залозі щурів, тоді як при введенні мультипробіотика «Симбітер® ацидофільний» патерн експресії більшості з досліджених генів подібний до контролю.*

Ключові слова: гіпоацидність, підшлункова залоза, експресія генів, мультипробіотики.

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Экспрессия генов *Cckbr, Gast, Reg1*α и *Tgfb1* в поджелудочной железе крыс в условиях длительной гипоацидности и при введении мультипробиотика «Симбитер® ацидофильный» концентрированный

Резюме

Цель. *Исследовать экспрессию генов Cckbr, Gast, Reg1*α *и Tgfb1 в поджелудочной железе крыс в условиях длительной гипоацидности, а также при введении мультипробиотика «Симбитер® ацидофильный» концентрированный.* ***Методы***. *Исследования проведены на белых нелинейных крысах-самцах. Состояние гипоацидности моделировали интраперитонеальным введением омепразола в течение 28 дней. Уровень экспрессии генов определяли полуколичественным анализом с помощью ОТ-ПЦР.* ***Результаты***. *Показано повышение уровня мРНК Cckbr, Reg1*α *и Tgfb1, а также появление экспрессии гена Gast в поджелудочной железе крыс в гипоацидных условиях. При введении мультипробиотика «Симбитер® ацидофильный» концентрированный в тех же условиях содержание мРНК Cckbr и Tgfb1 оказалось на уровне контрольных значений, экспрессия гена Gast не зафиксирована, а уровень мРНК Reg1*α *превышал этот показатель у животных с гипоацидным состоянием.* ***Выводы***. *Состояние длительной гипоацидности сопровождается изменением экспрессии генов Cckbr, Gast, Reg1*α *и Tgfb1 в поджелудочной железе, тогда как при введении мультипробиотика «Симбитер® ацидофильный» концентрированный паттерн экспрессии большинства исследованных генов близок к контролю.*

Ключевые слова: гипоацидность, поджелудочная железа, экспрессия генов, мультипробиотики.

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