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Реферати

УРОВЕНЬ ПЕРВИЧНЫХ ПРОДУКТОВ ПЕРОКСИДНОГО ОКИСЛЕНИЯ ЛИПИДОВ В УСЛОВИЯХ РАЗЛИЧНОЙ ФУНКЦИОНАЛЬНОЙ АКТИВНОСТИ СКЕЛЕТНЫХ МЫШЦ В СОЧЕТАНИИ С ИЗМЕНЕНИЯМИ ФОТОПЕРИОДА

Гильмутдинова М.Ш., Черно В.С., Кошарный В.В.

В статье рассматриваются особенности влияния иммобилизационного стресса и избыточных физических нагрузок в сочетании с изменениями фотопериода на уровень первичных продуктов пероксидного окисления липидов. Показано, что при сочетании иммобилизационного стресса с круглосуточным освещением, избыточных физических нагрузок с круглосуточным освещением, а также избыточных физических нагрузок со световой депривацией уровень первичных продуктов пероксидного окисления липидов повышается, что может свидетельствовать об усилении процессов пероксидации. Полученные результаты можно рассматривать как своеобразную адаптивную реакцию в исследуемых условиях.

Ключевые слова: пероксидное окисление липидов, иммобилизационный стресс, избыточная физическая нагрузка.

Стаття надійшла 11.07.18р.

THE PRIMARY PRODUCTS OF LIPID PEROXIDATION IN DIFFERENT FUNCTIONAL ACTIVITY OF SKELETAL MUSCLES IN COMBINATION WITH CHANGES IN PHOTOPERIOD

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The article considers the features of the influence of immobilization stress and excessive physical activity in combination with photoperiod changes on the level of primary products of lipid peroxidation. It is shown that the combination of immobilization stress with 24-hour lighting, excessive physical activity with 24-hour lighting, as well as excessive physical activity with light deprivation increases the level of primary products of lipid peroxidation, which may indicate the strengthening of peroxidation processes. The results can be considered as a kind of adaptive response in the conditions under study.

Key words: lipid peroxidation, immobilization stress, excessive physical activity.

РецензентКостенко В.О.

DOI 10.26724/2079-8334-2018-3-65-218-222
УДК 615.24:591.41/.434]:599.323.4

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IMPACT OF MULTIPROBIOTICS ON THE CONTENT OF TBA-REACTIVE SUBSTANCES IN THE BLOOD SERUM AND MUCOUS MEMBRANES OF THE STOMACH AND COLON IN RATS WITH LONG-TERM GASTRIC HYPOCHLORHYDRIA

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It has been established that after 28 days of administering omeprazole to rats, the TBARS content had grown in all the studied media: in the blood serum, by 92.1% ($p < 0.001$); in the gastric mucosa, by 352.4% ($p < 0.001$) and in the mucous membrane of the colon, by 57.3% ($p < 0.01$) compared to that of the control group. Under the conditions of the concomitant 28-day administration of omeprazole and Simbiter multiprobiotic, the TBARS content in the blood serum was the same as that in the control group rats. After 28 days of co-administering omeprazole and the “Apibact” multiprobiotic, the TBARS content in the blood serum was even by 41.4% ($p < 0.05$) lower than that in the control group. After 28 days of co-administering of omeprazole and Simbiter multiprobiotic, the TBARS content in the rat gastric mucosa was by 64.4% ($p < 0.01$) lower than in the group of rats given omeprazole only. In the concomitant administration of the “Apibact” multiprobiotic with omeprazole, the TBARS content in the gastric mucosa was by 57.8% ($p < 0.01$) smaller in comparison with the same value in the group of rats, which was administered omeprazole only for 28 days. "Simbiter" and "Apibact" multiprobiotics in the conditions of the 28-day concomitant administration with omeprazole reduced the TBARS content in the colon mucous membrane by 18.4% ($p < 0.05$) and 39.0% ($p < 0, 05$), respectively.

Key words: hypochlorhydria, probiotics, TBA-reactivesubstances (TBARS), mucous membrane of the stomach, mucous membrane of the colon.

The work is a fragment of the research project "The role of TRPV-4 receptors in the regulation of the digestive tract”, state registration number 0118U004306.

Prolonged reduction of hydrochloric acid gastric secretion can lead to a number of negative consequences, including deficiency of iron, calcium, vitamin B12, development of hyperhastrinemia,

microbiocenosis disorders in the digestive tract, and, consequently, development of inflammation and morphofunctional changes in the stomach [8] . One of the common mechanisms for the development of combined changes in the digestive system is an oxidative stress, which is based on free radical reactions that are universal. They provide the disease progression and the products of these reactions produce systemic effects on other organs. In this regard, they are considered as a factor of polymorbidity. In the free radical oxidation chain, the result of which is destruction of epithelial cells membrane lipoproteins, the first link is lipid peroxidation (LPO). The final product of LPO, malondialdehyde, being an integral part of TBA-reactivesubstances (TBARS), is the result of the formation and release of reactive oxygen intermediates (ROI). The most active of them is superoxide anion (O^{2-}), which in the dismutation reaction forms hydrogen peroxide H_2O_2. The latter enters into oxidative reactions with cell components that are distal from the place of H_2O_2 synthesis.

The purpose of the present study was to determine the content of TBA-reactive substances (TBARS) in the blood serum and in the mucous membranes of the stomach and the large intestine under the conditions of prolonged gastric hypoacidity and when Simbiter and Apibact multiprobiotics were administered.

Materials and methods. The study was performed on 40 white non-linear male rats with the initial weight of 160-180 g which were kept in the certified vivarium of the Educational and Scientific Center "Institute of Biology and Medicine" of the Taras Shevchenko National University of Kyiv in accordance with the "Standard rules for the ordering, equipping and maintenance of experimental biological clinics (vivaria) ". All experiments were carried out in accordance with the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruel Treatment". All animals were divided into 4 experimental groups. The first group of animals served as a control one. They were administered water for injections intraperitoneally (v / o) 0.2 ml daily and orally (0.5%) once a day for 28 days. Animals of the second group were administered omeprazole and 0.5 ml of water for injection orally once a day for 28 days. Animals of the third group were co-administered with omeprazole and Simbiter® acidophilic concentrated (Simbiter) multiprobiotic once a day for 28 days. Animals of the fourth group were co-administered with omeprazole and "Apibact®" (Apibact) multiprobiotic once a day for 28 days.

Omeprazole (manufactured by Sigma-Aldrich USA) was injected intravenously in the dose of 14 mg / kg, dissolved in 0.2 ml of water for injection. Simbiter and Apibact multiprobiotics (manufactured by “OD Prolisok”, Ukraine) were administered in combination with omeprazole orally at the dose of 140 mg / kg (1.4 * 1010 CFU / kg). Multiprobiotics were dissolved in 0.5 ml of water for injection.

The content of TBARS was estimated according to the method of Stalna, which lies in the fact that at the boiling temperature in acidic medium, malondialdehyde reacts with 2-thiobarbituric acid, thus forming a trimethine complex with a maximum absorption at λ = 532 nm. Aliquots of serum, cell suspension or mucosal homogenate (0.5 mg of protein) were placed in the buffer: 175 mM KCl, 25 mM Tris-HCl, pH = 7.4; the volume of the sample was 0.5 ml. The 20% trichloroacetic acid (TCA) in the amount of 0.2 ml was immediately added to the sample, the denatured protein was precipitated by centrifugation at 1000 g for 15 min. The 0.8% tiobarbituric acid (TBA) in the amount of 0.25 ml was added to 0.5 ml of the supernatant obtained, and the mixture was incubated in a boiling water bath for 10 minutes to develop the color. The color determination was carried out using a spectrophotometer (SF-46, LOMO, Russia) at l = 532 nm. The content of TBARS per 1 mg of protein was calculated based on the molar coefficient of the malonic dialdehyde complex extinction with 2-tiobarbituric acid: ε = 1.56 x 105 cm-1 x M-1. Statistical processing of the results was performed using the Statistica 7.0 software. Since the obtained data were normally distributed, we determined the mean value (M) and the standard error of the mean (m) and compared the samples using the Student's t-test.

Results of the study and their discussion. After 28 days of administering omeprazole to rats, the content of TBARS increased in all the studied media: in the blood serum, by 92.1% ($p < 0.001$); in the gastric mucosa, by 352.4% ($p < 0.001$) and in the mucous membrane of the colon, by 57.3% ($p < 0.01$) compared to the respective control. The increase of the TBARS content in the mucous membranes of the stomach and large intestine indicates a prooxidant-antioxidant balance disorder, which testifies to the development of inflammation in the organs under study. With the simultaneous 28-days administration of omeprazole and the Simbiter multiprobiotic, the TBARS content in the blood serum was the same as that in the control group rats. After 28 days of concomitant administration of omeprazole and the Apibact multiprobiotic, the TBARS content in the blood serum was even lower by 41.4% ($p < 0.05$) than that in the control group (figure 1). After 28 days of concomitant administration of omeprazole and Simbiter multiprobiotic, the TBARS content in the stomach mucous membrane of rats was 64.4% ($p < 0.01$) lower than that in the group of rats given omeprazole only.

Table 1

The TBARS content in blood serum and in mucous membranes of the stomach and colon in rats at 28-days omeprazole administration (M + m)

Medium	Control n = 10	Omeprazole n = 10
Blood serum	15.3±1.5	29.4±2.7***
Stomach mucous membrane	44.9±4.9	203.1±18.9***
Colon mucous membrane	130.3±11.2	205.0±19.1**

Note: ** - p<0,01, *** - p<0,001 compared to the control.

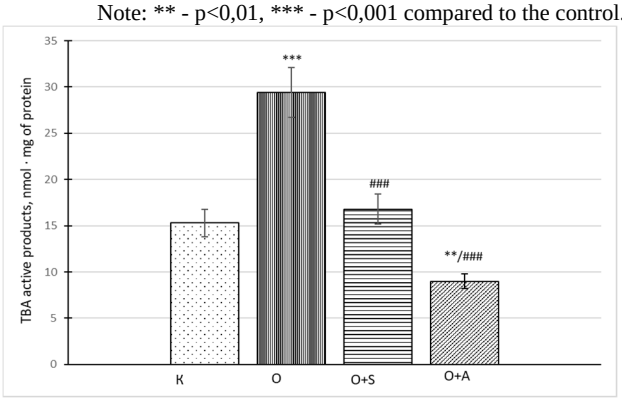

Fig. 1. The TBARS content in blood serum of rats за умов at 28-days combined omeprazole and multiprobiotics administration.

Note: ** - p<0,01, *** - p<0,001 compared to the control; ### - p<0,001 compared to the group of rats, which were administered omeprazole only.

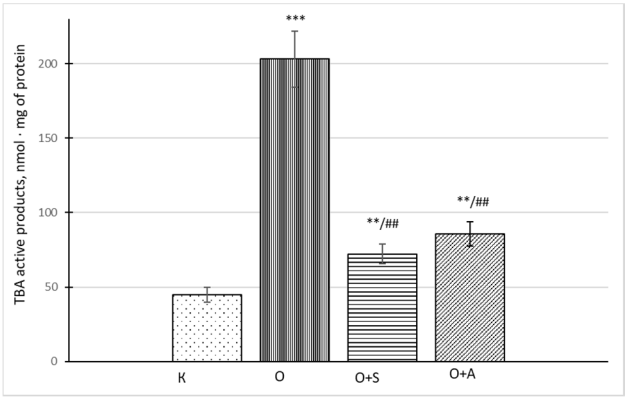

Fig. 2. The TBARS content in the rat stomach mucous membrane at 28-days concomitant omeprazole and multiprobiotics administration.

Note: p<0,05, ** - p<0,01, *** - p<0,001 compared to the control group; ## - p<0,01, compared to the group of rats, which were administered omeprazole only.

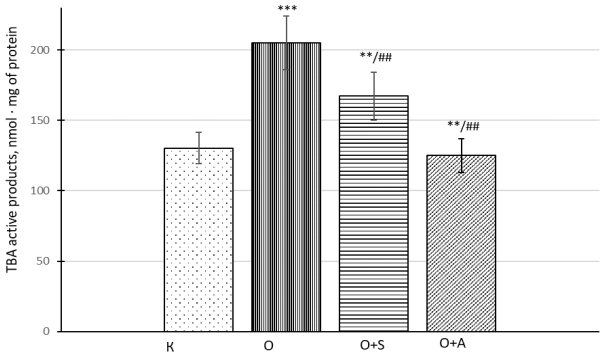

Fig. 3. The TBARS content in the rat colon mucous membrane at 28-days concomitant omeprazole and multiprobiotics administration.

Note: ** - p<0,01, *** - p<0,001 compared to the control group; # - p<0,05, ## - p<0,01 compared to the group of rats, which were administered omeprazole only.

Despite such a significant decrease, the TRARS content in the stomach mucous membrane did not reach control values and remained increased by 61.0% (p <0.01) compared to the control group.

Similarly to the "Simbiter" multiprobiotic, the TRARS content in the gastric mucosa was influenced by another probiotic drug, the "Apibact" multiprobiotic. In terms of its concomitant administration with omeprazole, the TBARS content in the gastric mucosa was by 57.8% (p <0.01) lower than that of the rats group, which received omeprazole only within 28 days, however it remained by 90.9% higher compared to that of the control group (p <0.01). There was no statistically significant difference between the effects of the multiprobiotics under study (figure 2)."Simbiter" and "Apibact" Multiprobiotics under the condition of 28-day concomitant administration with omeprazole reduced the TBARS content in the large intestine (colon) mucous membrane by 18.4% (p <0.05) and 39.0% (p <0,05), respectively. At the same time, in the group of rats, which were co-administered with omeprazole and Simbiter multiprobiotic, the TBARS content in the colon mucous membrane was by 28.4% (p <0.05) higher than that in the control group of rats, which were co-administered with omeprazole and Apibact multiprobiotic, the TBARS content in the colon mucosa was not statistically significantly different from that of the control group (figure 3). Comparison of the TBARS content in the blood serum and in the mucous membranes of the stomach and colon after co-administration of omeprazole and multiprobiotics showed that the least effect of multiprobiotics was on the TBARS content in the mucous membrane of the large intestine. It should be noted that the increase in the TBARS content in the colon mucous membrane after the 28-days administering of omeprazole only was the smallest. We associate it with the fact that the large intestine is the main microbiota reservoir of human organism in general and the digestive tract in particular.

In the biotope of the colon, representatives of 17 families, 45 genera and over 400 species of microorganisms were identified. All groups of microorganisms inhabiting the colon are divided into 3 groups. The first one is obligatory (dominant, main, indigenous, resident) (*Bifidobacterium, Lactobacillus, Propionibacterium, Bacteroides*), which performs the regulatory function and counteracts

the habitat colonization by random microorganisms and the excessive growth of opportunistic normobiota species. The second group is optional (extra, coexistent) (*Escherichia, Enterococcus, Fusobacterium, Clostridium, Eubacterium,* etc.), which synthesizes biologically active compounds, activates the immune system, participates in the metabolism of various compounds.

Most of the optional microorganisms are opportunistic species, which, in pathologically increasing populations, can cause serious complications of infectious nature. Therefore, their representation in healthy biocenosis is always limited in quantitative terms and is under constant control of the macroorganism and its friendly apathogenic microflora. The third group is the transient (allochthonous, random, residual) microflora (*Citrobacter, Enterobacter, Proteus, Klebsiella, Morganella, Hafnia, Staphylococcus, Pseudomonas, Bacillus,* etc.), among them there may be specimens with a high aggressive potential, which, at weakening protective functions of the obligated microflora, can increase the population and cause the development of pathological processes.

Therefore, the large intestine is more adapted to the quantitative and qualitative composition of microbiota. Due to it, the structural-functional changes in the colon are less pronounced than in the stomach, which is confirmed by a number of works, which show that after 28 days of omeprazole administering to rats, hyperplasia, dysplasia and metaplasia develop in the stomach [4], and, meanwhile, hyperplasia with signs of inflammation develops in the intestine [3, 9]. Thus, the less inflammation is, the less is the strength of oxidative stress. Therefore, the smallest TBARS content increase in the mucous membrane of the colon is the logical result.

Thus, the results obtained allowed to conclude: the "Apibact" multiprobiotic more efficiently influences on the TBARS content in the blood serum and in the mucous membrane of the large intestine. Taking into account that microorganisms used in multiprobiotics do not differ from each other by their qualitative and quantitative composition, we concluded that the stronger antioxidant action of the "Apibact" multiprobiotic is due to the presence of propolis in its composition, which has antioxidant properties due to its own composition [5]. The main chemical classes of substances represented in propolis are flavonoids, phenols and aromatic compounds [10]. Antioxidant action particularly inheres in flavonoids and phenols [7]. And the second important conclusion was made from the data obtained: the highest increase of the TBARS content after 28 days of the HCl secretion inhibition was observed in the mucous membrane of the stomach, indicating a higher intensity of the inflammatory process in the stomach compared with that in the colon. Obviously, this is the result of both dysbiotic changes in the stomach, which is normally poorly inhabited by microorganisms, and the proximity of target cells (ECL cells and parietal cells) to the gastrin, the long-term hypersecretion of which also causes the inflammatory process.

The literature analysis suggests that the data obtained are expected regarding the antioxidant properties of multiprobiotics. After all, the phenomenon of the LPO process inhibition by different strains of lactic acid bacteria is described in a number of papers [6]. In addition, it has been shown that the “Apibact” multiprobiotic reduces the LPO products content in the pancreas and liver of rats at the long-term gastric juice hypoacidity caused by omeprazole [1, 2].

It is important to note that in the treatment of gastro-esophageal reflux disease, omeprazole is prescribed for at least one month of daily intake, but there is no widespread use of probiotics in the complex treatment of this disease, as opposed to *H. pylori*-associated gastritis and ulcers.

Conclusions

1. Prolonged gastric hypoacidity led to intensification of the lipid peroxidation reaction, which was manifested in the increase in the TBARS content in all the studied media: in the blood serum, by 92.1% ($p < 0.001$); in the mucous membrane of the stomach, by 352, 4% ($p < 0.001$) and in the mucous membrane of the colon, by 57.3% ($p < 0.01$).
2. Long-term administration of multiprobiotic drugs against the background of gastric hypochlorohydria significantly reduced the manifestation of inflammatory process in the mucous membranes of the stomach and colon, which was manifested as the TBARS content reducing in the studied rat media.

The prospect of further research in this field is the study of multiprobiotics effect on the TBARS content in the organs of the digestive tract in rats under the conditions of other pathologies.

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Реферати

ВПЛИВ МУЛЬТИПРОБІОТИКІВ НА ВМІСТ ТБК-АКТИВНИХ ПРОДУКТІВ У СИРОВАТЦІ КРОВІ ТА СЛИЗОВИХ ОБОЛОНКАХ ШЛУНКУ І ТОВСТОЇ КИШКИ ЩУРІВ З ТРИВАЛОЮ ШЛУНКОВОЮ ГІПОХЛОРГІДРІЄЮ

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Встановлено, що після 28-ми днів введення щурам омепразолу вміст ТБК-активних продуктів зростав у всіх досліджуваних середовищах: в сироватці крові – на 92,1% ($p<0,001$), в слизовій оболонці шлунку – на 352,4% ($p<0,001$) і в слизовій оболонці товстої кишки – на 57,3% ($p<0,01$) у порівнянні з відповідним контролем. За умов одночасного 28-ми денного введення омепразолу та мультипробіотика «Симбітер» вміст ТБК-активних продуктів в сироватці крові був таким же, як і у щурів контрольної групи. Після 28-денного сумісного введення омепразолу та мультипробіотика «Апібакт» вміст ТБК-активних продуктів в сироватці крові був навіть на 41,4% ($p<0,05$) меншим, ніж в контролі. Після 28-ми денного сумісного введення омепразолу і мультипробіотика «Симбітер» вміст ТБК-активних продуктів в слизовій оболонці шлунка щурів був на 64,4% ($p<0,01$) меншим, ніж в групі щурів, яким вводили один омепразол. За умов сумісного введення мультипробіотику «Апібакт» з омепразолом вміст ТБК-активних продуктів в слизовій оболонці шлунка був на 57,8% ($p<0,01$) меншим у порівнянні з аналогічним показником в групі щурів, яким упродовж 28-ми днів вводили один омепразол. Мультипробіотики «Симбітер» і «Апібакт» за умов 28-ми денного сумісного введення з омепразолом знижували вміст ТБК-активних продуктів в слизовій оболонці товстої кишки на 18,4% ($p<0,05$) і 39,0% ($p<0,05$), відповідно.

Ключові слова: гіпохлоргідрія, пробіотики, ТБК-активні продукти слизова оболонка шлунка, слизова оболонка товстої кишки.

Стаття надійшла 6.06.18 р.

ВЛИЯНИЕ МУЛЬТИПРОБИОТИКОВ НА СОДЕРЖАНИЕ ТБК-АКТИВНЫХ ПРОДУКТОВ В СЫВОРОТКЕ КРОВИ, А ТАКЖЕ СЛИЗИСТЫХ ОБОЛОЧКАХ ЖЕЛУДКА И ТОЛСТОЙ КИШКИ КРЫС С ДЛИТЕЛЬНОЙ ЖЕЛУДОЧНОЙ ГИПОХЛОРГИДРИЕЙ

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Установлено, что после 28-ми дней введения крысам омепразола содержание ТБК-активных продуктов возросло во всех исследуемых средах: в сыворотке крови – на 92,1% ($p<0,001$), в слизистой оболочке желудка – на 352,4% ($p<0,001$) и в слизистой оболочке толстой кишки – на 57,3% ($p<0,01$) по сравнению с контролем. В условиях одновременного 28-ми дневного введения омепразола и мультипробиотика «Симбитер» содержание ТБК-активных продуктов в сыворотке крови было таким же, как и у крыс контрольной группы. После 28-дневного совместного введения омепразола и мультипробиотика «Апибакт» содержание ТБК-активных продуктов в сыворотке крови было даже на 41,4% ($p<0,05$) меньшим, чем в контроле. После 28-ми дневного совместного введения омепразола и мультипробиотика «Симбитер» содержание ТБК-активных продуктов в слизистой оболочке желудка крыс было на 64,4% ($p<0,01$) меньшим, чем в группе крыс, которым вводили один омепразол. При ссовместном введении мультипробиотика «Апибакт» с омепразолом содержание ТБК-активных продуктов в слизистой оболочке желудка был на 57,8% ($p<0,01$) меньшим по сравнению с аналогичным показателем в группе крыс, которым на протяжении 28-ми дней вводили один омепразол. Мультипробиотики «Симбитер» и «Апибакт» в условиях 28-ми дневного совместного введения з омепразолом понижали содержание ТБК-активных продуктов в слизистой оболочке толстой кишки на 18,4% ($p<0,05$) и 39,0% ($p<0,05$), соответственно.

Ключевые слова: гипохлоргидрия, пробиотики, ТБК-активные продукты, слизистая оболочка желудка, слизистая оболочка толстой кишки.

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