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Influence of multiprobiotic Symbiter® acidophilic concentrated on pro- and antioxidant balance in rat liver during long-term gastric hypoacidity

Wpływ stężonego multiprobiotyku *Symbiter® acidophilic* na równowagę pro- i antyoksydacyjną w wątrobie szczurów w przebiegu długoterminowej niedokwasoty żołądka

INTRODUCTION

Pathological conditions of the upper gastrointestinal system, which are accompanied by a reduced synthesis of hydrochloric acid (atrophic gastritis, chronic giant hypertrophic gastritis, long-term treatment of antisecretory drugs), lead to morpho-functional and microecological violations in the gastrointestinal tract [1, 10]. Gastric hypochlorhydria reduces the stimulating effect of cholecystokinin and secretin (which are excreted to the duodenum under the influence of hydrochloric acid) on bile secretion. The above violations may result in the development of pathological processes in the hepatobiliary system, one indicator of which is violation of the pro/antioxidant status of liver cells. To restore the oxidative-antioxidant balance it is necessary to replenish stores of intracellular antioxidants. It is known that probiotics have antioxidant properties [6]. We used multiprobiotic Symbiter® acidophilic concentrated (Symbiter) – the concentrated biomass of living cells of multicomponent symbiotic probiotic bacteria (Bifidobacterium, Lactobacillus, lactic streptococci and propionic acid bacteria).

The aim was to investigate the effect of multiprobiotic Symbiter on oxidation-antioxidant balance in rat liver during long-term inhibition of gastric secretion.

MATERIAL AND METHODS

Studies were carried out on white non-linear mature male rats. Hypoacidity state was simulated using intraperitoneal administration of omeprazole (14 mg/kg) (Sigma, USA) once a day during 28 days. Rats in the 2nd group simultaneously with the introduction of omeprazole received multiprobiotic Symbiter (produced by LLC "OD Prolisok") orally at a dose of 0.14 ml/kg, dissolved in 0.5 ml of water for injection, once a day during 28 days.

Lipids from rat liver were extracted using the Folch method by chloroform-methanol mixture (2:1, v/v) [3]. From these total lipid shot spectra in the infrared (IR) Fourier spectrometer "Nexus" ("Thermo

Nicolet", USA), equipped with sensor DTGS. Measurement of peak areas in the IR spectra was carried out using the software appliance OMNIC (version 5.1). Quantitative evaluation of the structural elements of lipids was made using the method of valuation areas, and determined their percentage [7]. The content of diene conjugates was determined in heptane-izopropanol extract spectrophotometrically, and Schiff bases – with the help of the fluorimetric method [4]. The content of TBA-active compounds was determined by the reaction with thiobarbituric acid [9]. Assessment of superoxide dismutase activity (SOD) was performed using nitro tetrazolium [2], and evaluation of catalase – by reduction of the amount of H_2O_2 in solution after incubation in optimal conditions [5]. Statistical processing of results of research was carried out using standard methods of variation statistics [8].

RESULTS

It is shown that prolonged inhibition of gastric secretion of hydrochloric acid by omeprazole caused significant changes in the structural organization of rat liver lipids (Table 1). We observed increased content of hydroxyl groups – 2.4 times; of aldehydic groups for – 1.7 times, of phosphate groups – 1.4 times and of trans-isomers 1.2 times in comparison with control. Under these same conditions we fixed a decrease of carbonyl groups and cis-isomers for 1.3 and 1.5 times, respectively.

At combined introduction of animal Symbiter with omeprazole, we fixed significant restoration of structural organization of liver lipids compared with that in a group of rats with hypoacidity state. We also fixed a decrease of the levels of hydroxyl groups (1.4 times), aldehyde groups (1.5), phosphate groups and trans-isomers (1.2 times), and an increase of content of CH-groups (1.4 times), carbonyl groups and cis-isomers (1.2 times) relative to the group of rats injected with omeprazole (Table 1).

The study on the content of lipid peroxidation products in rat liver showed that after prolonged oppression of secretion of HCl their level increased: conjugated dienes – 2.2 times, TBA-active compounds – 1.7 times, and Schiff bases – 1.6 times in comparison to control (Table 2). Simultaneous introduction of Symbiter and omeprazole for 28 days decreased the content of the compounds: conjugated dienes – 1.4 times, TBA-active compounds – 1.3 times, and Schiff bases – 1.2 times in comparison to the animals with hypoacidity state (Table 2).

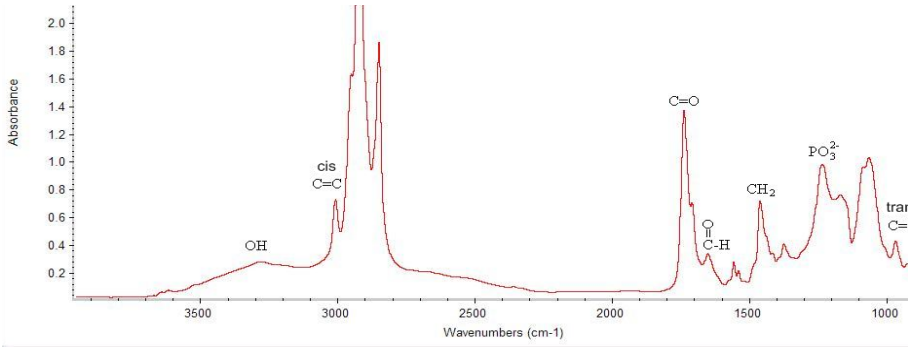

Fig. 1. IR Fourier spectrum of total lipids of liver of control rats

Table 1. The number of functional groups (% of total) in the lipids of rat liver during long-term gastric hypoacidity (M ± m, n = 10)

Examined parameter / Group of animals	Control	Omeprazole	Omeprazole + Symbiter
Hydroxyl groups (3600-3100 cm^{-1})	11.06 ± 1.01	26.61 ± 1.85*	16.93 ± 1.52#
Cis-isomer (3010 cm^{-1})	2.65 ± 0.12	1.71 ± 0.09*	2.07 ± 0.14#
CH group (2800-2929 cm^{-1})	45.95±2.53	32.81±2.09*	44.26± 2.15#
Carbonyl group (1720 cm^{-1})	22.28 ± 1.92	16.06 ± 1.47*	18.52 ± 1.63#
Aldehyde groups (1679 cm^{-1})	1.67 ± 0.09	2.91 ± 0.11*	1.37 ± 0.12#
CH_2 group (1454 cm^{-1})	6.95±0.70	6.87±0.81	6.74±0.42
Phosphate groups (1210 cm^{-1})	7.78± 0.84	11.02± 1.03*	8.51± 0.69#
Trans-isomers (968 cm^{-1})	1.66 ± 0.08	2.01 ± 0.13*	1.60 ± 0.07#

* $P \leq 0.05$ in comparison to control # $P \leq 0.05$ as compared to the group of animals injected with omeprazole

Table 2. The content of lipid peroxidation products in rat liver during long-term gastric hypoacidity (M ± m, n = 10)

Examined parameter / Group of animals	Control	Omeprazole	Omeprazole + Symbiter
Diene conjugates. nmol x mg $protein^{-1}$	290.69 ± 19.46	632.71 ± 37.37*	401.13 ± 29.85#
TBA-active compounds. nmol x mg $protein^{-1}$	74.81 ± 5.58	125.81 ± 11.43*	87.57 ± 5.55#
Schiff base. con. un. x mg $protein^{-1}$	8.65 ± 0.55	13.43 ± 0.85*	10.21 ± 0.56#

* $P \leq 0.05$ in comparison to control; # $P \leq 0.05$ as compared to the group of animals injected with omeprazole

Table 3. The activity of antioxidative enzymes in rat liver during long-term gastric hypoacidity (M ± m, n = 10)

Examined parameter / Group of animals	Control	Omeprazole	Omeprazole + Symbiter
Superoxide dismutase, con. un. x min^{-1} x mg $protein^{-1}$	2.69 ± 0.24	2.23 ± 0.19*	2.81 ± 0.21#
Catalase, nmol x min^{-1} x mg $protein^{-1}$	69.63 ± 5.61	52.79 ± 4.93*	62.98 ± 4.38#

* $P \leq 0.05$ in comparison to control; # $P \leq 0.05$ as compared to the group of animals injected with omeprazole

In the study of enzyme front line protecting cells from reactive oxygen forms (ROS) of superoxide dismutase and catalase it was determined that a prolonged influence of omeprazole on rat liver reduced their activity 1.2 times in comparison to the control group of animals (Table 3). The joint introduction of Symbiter and omeprazole increased superoxide dismutase activity in hepatocytes 1.3 times, and catalase 1.2 times in comparison to the group of animals that received only omeprazole.

DISCUSSION

Prolonged gastric hypochlorhydria is characterized by changes in the composition and the rate of oxidative changes in membrane lipids of rat liver. We observed increased levels of hydroxyl, aldehyde groups, trans-isomer and a decrease of the content of cis-isomers, which may indicate the activation of free radical processes. The observed reduction of carbonyl, CH-groups and the increase of phosphate groups may be indicative of increased metabolism in the phospholipids membranes of hepatocytes. The observed increase in the number of formed lipid peroxidation products (conjugated dienes, TBA-active compounds, Schiff bases), as well as decreased activity of antioxidant enzymes (superoxide dismutase and catalase) in liver cells of rats with hypoacidity condition testified to violation of the balance between ROS production and neutralization.

Thus, prolonged gastric hypochlorhydria in the liver of rat causes a shift of the oxidation-antioxidant balance towards the intensification of free radical oxidation of lipids, which in turn is an indicator of the development of oxidative stress in hepatocytes. Multiprobiotic Symbiter partially restores the impaired pro/antioxidant balance in the liver of rats with prolonged suppression of secretion of HCl.

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SUMMARY

It was shown that prolonged inhibition of gastric secretion of hydrochloric acid by omeprazole, significant changes in the structural organization of lipids are observed in rat liver as well as increased content of lipid peroxidation products, indicating activation of free radical processes in hepatocytes. Multiprobiotic Symbiter normalised research indicators in liver of rats with hypoacidity state.

Key words: multiprobiotic, Symbiter® acidophilic, rat, liver, pro- and antioxidant balance, gastric hypoacidity

STRESZCZENIE

Wykazano, że w efekcie przedłużonego hamowania wydzielania żołądkowego kwasu solnego omeprazolem w wątrobie szczurów dochodzi do istotnych zmian w organizacji strukturalnej lipidów, wzrostu zawartości produktów peroksydacji lipidów, co wskazuje na aktywację procesów wolnorodnikowych w hepatocytach. Multiprobiotyk Symbiter® normalizuje badane wskaźniki w wątrobie szczurów z niedokwasotą.

Słowa kluczowe: multiprobiotyk, Symbiter® acidophilic, szczury, wątroba, równowaga pro-/antyoksydacyjna, niedokwasota żołądka