Multiprobiotic Administration Induced Longevity and Decreased Oxidative Stress in Rats.

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ABSTRACT

Considering free radical and Mechnikovs' theories of aging we decided to investigate the influence of multiprobiotic on life span and concentration of lipid peroxidation products in serum and tissues of rats. The study was carried out on 300 white Wistar rats. Rats were divided into 2 groups - control and experimental. Rats of experimental group were treated with multiprobiotic «Symbiter» acidophilic concentrated per os periodically along the life. The survival rate of rats was calculated by Kaplan-Meier test, and differences in survival rates were tested for significance by the log rank test. At the age of 3, 6, 12, 18, 21 and 24 months in each group 10 rats were decapitated and content of conjugated dienes, Schiff bases, thiobarbituric acid reactive substances, superoxide dismutase, catalase were measured in blood serum, gastric mucosa, pancreas and hepatocytes. It was established that periodic multiprobiotic administration increased life span of rats, prevented increase of lipid peroxidation products concentration in blood serum and other tissue and normalized activity of antioxidant ferments.

Keywords: life span, multiprobiotic, lipid peroxidation.

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INTRODUCTION

Nowadays gerontology had already proved that in the process of aging a lot of change occur in different structures and functions of organism. There are more than 300 theories of aging. Mechnikov I.I. suggested one of the first theories [1]. He distinguished two types of aging physiological and pathological, and paid attention both to biological and social factor of this process. According to Mechnikov, during aging, products of nitrogen metabolism (ammonia) accumulate and under the influence of products of decomposition the process of intoxication and auto intoxication intensifies. According to this theory, intoxication affects specific parenchymal tissue (hepatic and brain cells). On the contrary, connective tissue cells hypertrophy and replace dead cells of vital organs. Mechnikov I.I. considered age-related atrophy as process of phagocytosis and sought the way to preserve specific parenchymal cells [1].

Another prominent theories to explain ageing is the free radical theory of ageing. The theory was suggested by Denham Harman in the 1950s. He proposed that free radicals are responsible for damage associated with ageing. The antioxidant systems are unable to counterbalance all the free radicals continuously generated during the life of the cell. This results in oxidative damage in the cell and thus in tissues. Many works have experimental proofs in support of this theory [2]. Today other view on free radical theory had appeared. Y. Edrey and A. Salmon showed that in transgenic mice modulation of antioxidant expression didn’t generally affected mouse lifespan, but had significant influence on development of age-related pathologies [3,4].

The positive effect of probiotics on the functioning of various systems in the body have already proved by numerous studies. To halt the process of decay in the gut Metchnikoff suggested to use cultured dairy products that createa hostile environment for put reative bacteria. In old mice in comparison with young the reduction of nitric oxide, cytokines IL-6 and TNF-α were shown, also secretion of immune suppressive prostaglandin E2 (PGE2) was increased by peritoneal and spleen macrophages. Proliferation of stimulated splenocytes and IL-2 production were decreased in old mice, on the contrary secretion of IL-6 and TNF-α were increased. Feeding of old mice with La-Dahi or LaBb-Dahi probiotics improved function of peritoneal macrophages. Stimulation of nitric oxide, IL-6 synthesis and decreasing in PGE2 production proved this fact. La-Dahi or LaBb-Dahi probiotics consumption by old mice also stimulated proliferation and production of IL-2, in such way the functions of lymphocytes were enhanced [5]. Authors concluded that La-Dahi or LaBb-Dahi probiotics were effective in the reversal of age-dependent decrease in immune function in mice. Other probiotic stains such as L. acidophilus and B. lactis prevented and decreased age related metabolic dysfunction [6].

In our work we tried to create experimental model of animal aging that investigated influence of probiotic on rats’ organism. Thus, the aim of the study was to investigate the influence of multiprobiotic “Symbiter® acidophilic” concentrated (MP) administration on life longevity, lipid peroxidation and activity of antioxidant enzymes in different tissues of rats.

MATERIALS AND METHODS

Animals

The study was carried out on 300 white non linear rats in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the general ethical principles of animal experiments, approved by the First National Congress on Bioethics Ukraine (September 2001). The protocol was approved by the Committee on the Ethics of Animal Experiments of the Taras Shevchenko National University of Kyiv (Protocol number: 14/2014). The rats were kept in collective cages in controlled conditions of temperature (22±3°C), light (12h light/dark cycle) and relative humidity (60±5%). The animals were fed laboratory chow and tap water ad libitum.

Animal grouping

Rats were divided into 2 groups - control and experimental. Rats of experimental group in the first 10 days after birth got 1 drop of MP daily. Subsequently, these rats at the age of 3, 6, 9, 18, 21 and 24 months during the 30 days were administered orally MP (14 mg/kg) per os diluted in tap water (100 μl per 100 g of...
animal body weight). Control animals were administered at the same time dechlorinate tap water (100 µl/100 g).

One dose of MP (10 ml) consist of biomass of 14 living probiotic stains in symbiosis: Lactobacillus and Lactococcus – non less than $6.0 \times 10^{10}$ CFU/cm$^3$, propionic-acid bacteria –$3.0 \times 10^{10}$ CFU/cm$^3$, bifidobacteria – $1.0 \times 10^{10}$ CFU/cm$^3$, acetic-acid bacteria – $1.0 \times 10^{6}$ CFU/cm$^3$. Scientific and Production Company “O.D. Prolisok” (Kyiv, Ukraine) supplied MP used in this study.

**Life span assay**

The date of birth of each rat was recorded to study the longevity and survival. Assessment of the overall health of rats and fixation of their deaths were carried out every day. Animals that were sacrificed to study the biochemical parameters were excluded from the analysis of longevity. The survival rate of rats was calculated by Kaplan-Meier test, and differences in survival rates were tested for significance by use of the log rank test[7].

**Biological material and the study of oxidative-antioxidant system**

At the age of 3 month (juvenile age), 6 month (young age), 12, 18 month (adult age), 21 month (eldery) and 24 month (senile animals) old in each group 10 rats were decapitated. After sacrifice blood was collected from the heart into centrifuge tubes without anticoagulant and leaved for 20-30 minutes at room temperature to complete the formation of a clot. Then, blood samples were centrifuged at 1000 g for 15 minutes, and the supernatant (serum) was harvested in separate disposable microtubes.

The stomachs were harvested, opened along the lesser curvature and were treated with cold saline. **Gastric mucosa** was removed by surgical instruments in petrie dish. With use of 0.1M phosphate buffer (pH=7.4) and Potter homogenizer 10% homogenates were prepared. Material was filtered through a nylon filter to remove not completely destroyed cells and nuclei. Loose sediment discarded. The supernatant was harvested in separate disposable microtubes. All manipulations associated with the collection of material and tissue homogenates preparation carried out at temperatures from 0 to +4°C. **Pancreas** homogenates was prepared in same way.

Non-enzymatic method of hepatocytes isolation was used. First, hepar was resected. Than tissue sample (350 mg) was perfused with Hanks’ salt solution (HSS) and grind through four layers of nylon mesh in 10 ml of HSS. Samples were centrifuged (600 g, 3 min), precipitate was washed twice under the same conditions. Received liver cells were resuspended in HSS to get concentration of $2-3 \times 10^7$ cells/ml. Obtained samples were harvested in separate disposable microtubes. Microtubes with all kind of biological materials were frozen at -20°C[8].

The concentration of conjugated dienes (CD) was measured spectrophotometrically in heptane-isopropanol extract (Gavrilov method), content of Schiff bases (SB) - by fluorometric method of Kolesova, tiobarbituric acid reactive substances (TBARS) - by reaction with thiobarbituric acid (Stalnoy method) [9–11].

Superoxide dismutase (SOD) activity was determined by Chevari method (with use of nitroblue tetrazolium). Catalase (CAT) activity assessment was carried out by spectrophotometric analysis of the rate of $H_2O_2$ decomposition (Korolyuk method )[12,13].

**Statistical analysis**

Statistical analysis of data was carried out by the ”Statistica 8.0” software package. Due to the small sample size, for the investigation of the data distribution type Shapiro-Wilk’s W criterion was used. Probability of a type I error was $\alpha$ $>0.05$. Post-hoc analysis included Student’s t-test for parametric data. Mean of value (M) and standard error of the mean (m) were calculated as all datas were parametric.

**RESULTSAND DISCUSSION**

It was established the mean life span of control rats was $550.50 \pm 18.82$ days. In experimental group it was $11.9\%$ ($p<0.05$) greater and was equal to $616.48 \pm 23.08$ days (fig.1).
Administration of Symbiter increased the average life span compared to control group. Based on the rats survival assay, we can conclude that in our study multiprobiotic induced significant anti-aging effect. Our results are supported by works of other scientists. Sohal and Weindruch showed that various probiotics are known to extend life span and to retard age-related health declines in a number of different species, including rodents, worms, yeast and possibly primates[14]. Also such effectively increased the mean life span of C. elegans was demonstrated by Yang Zhao and his colleagues. They showed that the life-prolonging rate reached a maximum of 11.9% in C. elegans and suggested that different bacterial strains exert diverse life-prolong effect, and it is necessary to separately investigate the anti-aging effects of a new strain[15]. The reason why intestinal microbiota might extend lifespan was named by Mitsuharu Matsumoto. He suggested that intestinal microbiota stimulates host mucosal and systemic immunity[16].

In our work, we made conclusion that concentration of CD increased in blood serum, hepatocytes and pancreas with age (tab.1). Content of CD in blood serum increased in senile rats (24 month). In 24 month the value significant increased by 56.7% (p<0.05) compared to 3 month old rat. In hepatocytes CD had been significant increasing since 6 month age. It increased by 21.9% (p<0.05), 45.1% (p<0.05), 67.8% (p<0.05), 51.7% (p<0.05) and 74.9% (p<0.01) correspondently in 6, 12, 18, 21 and 24 month in comparison with young rats. In pancreas accumulation of CD was recorded in 12, 21 and 24 month. Concentration of CD in gastric mucosa hadn’t significant change with age.

In numerous works it was proved that lipid peroxidation (LP) is a process of lipid degradation due to the free radical and is the reason of cell degradation[17]. Also scientists have already proved that with age oxidative stress appears in different tissues of organism. For example, Dipanshu Kumar Vishwas and his colleagues have already shown that in adult and old hamsters concentration of TBARS in blood serum, spleen and bone marrow increased compared to young animals [18]. Çoban J showed that DC levels were higher in tissues of aged rats than young rats [19]. Our results are consistent with above mentioned authors.

It was estimated that SB in all tissues were greater in old rats compared to young. Content of SB in 6, 12, 18, 21 and 24 month old rats increased in blood serum correspondently by 48.8% (p<0.05), 129.4% (p<0.05), 90.5% (p<0.05), 147.8% (p<0.05), 264.7% (p<0.01) compare to juvenile rats. Increase of SB in gastric mucosa also was observed beginning from 6 month. The value have changed by 27.6% (p<0.05), 62.3% (p<0.05), 44.5% (p<0.05), 196.5% (p<0.01) correspondently in 6, 12, 18, 21 and 24 month old rats in comparison with 3 month. Contrariwise in hepatocytes and pancreas SB increased only in 21 and 24 month.

With age concentration of TBARS increased in all tissues. In blood serum, pancreas and gastric mucosa data began to increase in 18 month, in hepatocytes such changes had happened earlier in 12 month.
Our results have showed that with age intensity of oxidative stress increased in all examined tissues. As it been mentioned above, CD didn’t change with age in gastric mucosa. We consider that intensity of peroxidation explain such situation. Formation of secondary and final products of lipid degradation was such vigorous that primary products was few to reflect. Nowak M described similar situation [20].

Table 1: The content of lipid peroxidation products and activity of antioxidant ferments in different tissues

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In our previous works that were concerning long-term hyperacidity the properties of probiotics to decrease intensity of lipid peroxidation in different tissues of rats, eliminate disbacteriosis was described [21,22]. Taking into account these results, we decided to research the ability of MP to normalize activity of SOD, CAT and influence on LP.

In senile rats of experimental group content of CD in blood plasma, hepatocytes and pancreas was at the same level as in 3 month old control rats, in gastric mucosa CD concentration decreased by 35.9% (p<0.05). Moreover in blood serum CD at 6, 12, 18, 21 month was even lower than in juvenile control.
The same as in control group, concentration of SB and TBRAS increased in all tissues in the process of aging, but in experimental group, the intensity of lipid peroxidation was lower. SB in blood serum, hepatocytes, pancreas and gastric mucosa of 24 month old experimental rats decreased by 32.3% (p<0.05), 33.7% (p<0.05), 30.9% (p<0.05), 35.3% (p<0.01) correspondently compare to control of same age group. In multiprobiotic group significant increase of TBRAS in blood serum, pancreas and gastric mucosa was observed only since 18 month. Till that time TBRAS, in above mentioned tissues, was lower or didn’t significant differ from value of 3 month old control. Despite increase in 18 month the data of experimental group remained lower than control. For example in blood serum of 24 month old rats value decreased by 38.6% (p<0.01) in comparison with same aged control.

The increase of LP was accompanied with disturbance in functioning of antioxidant system. It was established that in blood serum SOD decreased by 25.2% (p<0.05) at 24 month compare to 3 month, in other age group data hadn’t significant differ. In 12, 18, 21 and 24 month SOD decreased in hepatocytes by 17.8% (p<0.05), 28.5% (p<0.05), 80.8% (p<0.05), 87.9% (p<0.01) correspondently compare to 3 month. Activity of SOD in pancreas and gastric mucosa hadn’t significant change with age.

CAT activity in blood serum significant decreased by 42.3% (p<0.05), 36.4% (p<0.05), 81.1% (p<0.05), 93.0% (p<0.05) correspondently in 12, 18, 21 and 24 month compare to 3 month. In gastric mucosa decrease of CAT also was determine since 12 month. On contrary in hepatocytes and pancreas significant difference was fixed only in eldery and senile group.

We suggested that decrease of CAT and SOD activity the same as increase of content of lipid peroxidation products proved the presence of oxidative stress in all tissues. We assumed in pancreas and gastric mucosa decrease of CAT and unchanged of SOD indicated defection of antioxidant system of cells.

Also, it was established that MP administration prevents age related changes in antioxidant system. CAT activity in hepatocytes of experimental rats in 21, 24 month increased by 22.3% (p<0.05), 30.6% (p<0.05) correspondently compare to control group of same age and these values didn’t significant differ from 3 month old control. Similar changes was observed in pancreas. In 24 month CAT increased by 19.7% (p<0.05) compare to same age group. In gastric mucosa of experimental rats CAT decreased only in 24 month. In blood serum CAT in 6, 12 was higher than 3 month control, in 18, 21, 24 CAT decreased.

MP consumption change both CAT and SOD activity. SOD content in blood serum of young, adult and eldery MP rats was higher than in control animals of same age, but in 24 month such positive tendency was diminished. Similar results we obtained in hepatocytes. In 6, 12, 18 month in experimental group SOD activity was correspondently increased by 53.6% (p<0.05), 72.7% (p<0.05), 22.0% (p<0.05) compare to control group of same age.

Our results showed that periodic MP administration caused an increase in antioxidant ferment activity, which has the potential to delay aging by counteracting the impact of free radicals. Such conclusion also was made by Yang Zhao[15]. Moreover Yael H. Edrey, Adam B. Salmon supposed that altering expression of antioxidant genes in mice does affect the aging process, but has limited effects on longevity[3].

CONCLUSION

Periodic multiprobiotic administration prevented the development of oxidative stress due to decrease of lipid peroxidation in blood serum, gastric mucosa, pancreas and hepatocytes and normalization of antioxidant ferments activity. Such systemic effect increased life span of rats.

REFERENCES