The Role of Oxidative Stress in Digestive Tract Morbidity

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The aim of the work was to evaluate changes in the peroxidative-antioxidative state of the organism induced by ulcer, gastritis, duodenitis and ulcerative colitis. The study cohort comprised 43 patients and 28 healthy subjects. Malondialdehyde concentration and catalytic activity in the blood serum of patients were compared with the same indices of healthy subjects. In ulcer and gastritis patients, lipid peroxidation was found to be more intensive and catalytic activity lower than in healthy controls. In ulcerative colitis patients the intensity of lipid peroxidation was decreased and catalysis was more intensive. The antioxidative system of the organism seems to be powerful enough for the enzymes of this system to neutralize the formation of free oxygen radicals.

Key words: lipid peroxidation, antioxidant concentration, malondialdehyde, catalase, free radicals, oxidative stress

INTRODUCTION

Over the recent years, the important role of lipid peroxidation (LP) in the formation of a pathology in the organism has been recognized. Derangements in LP regulation have been proved to be related with LP intensification and exhaustion of the antioxidative system of the organism, resulting in a number of clinical states (1). Peroxidation products begin accumulating in the cell structures and the surrounding intracellular liquids. They cause changes in the intracellular structures containing much proteins, lipids and phospholipids which contain easily peroxidized unsaturated fatty acids. The above-mentioned structures are cytoplasmic memranes consisting of phospholipids and various proteins – enzymes, antibodies, antigens. Thus, LP and antioxidation are the key processes that take part in maintaining the functional stability of membranes in the course of normal physiological processes. When the physiological status of the organism is normal, LP is strictly controlled by the antioxidative systems in which enzymes as superoxide dismutase, catalase, glutathionperoxidase, glutathionreductase play a pivotal role (2).

These enzymes in human cells neutralise free radicals (the radical form of reactive oxygen) which perform intensively in LP processes in the organism. Reactive oxygen becomes involved in cell growth, its division, progression, and death. At low concentrations these enzymes are indispensable for the normal processes to occur, while their higher concentrations play an important role in the development of various diseases (3). A number of enzymic and non-enzymic antioxidants protect the organism against accumulation of reactive oxygen particles (4). The antioxidant probucol exhibits this type of activity. In the presence of gastric mucosa damages, probucol protects the cells from acute affections and helps to cure gastric ulcers by inhibiting the formation of malondialdehyde (5).

The antioxidative system is one of the adaptive systems of the organism. It regulates the LP processes. Under the long-lasting effect of an irritant, the enzymes of this system become less active, and many toxic compounds – various peroxides, free radicals – apear (4), changing cell membrane permeability in the mucous membrane. The result is inflammation and disturbed functions of some organs. LP is disturbed in patients with digestive tract (6, 7), renal (8), cardiovascular (9), oncological (10) diseases, diabetes (11, 12).

Surplus free radicals in the cell cause an oxidative stress in the organism (13); the indices characterizing the peroxidative and antioxidative status of the organism undergo changes (14).

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With increasing the content of LP and dienic conjugates (DC) in biological liquids, the concentrations of a-tocopherol, ascorbic acid in the organism become lower (15), therefore the influence of the antioxidative system on the pathogenesis of gastroenterological diseases is proved also by a positive effect of natural and synthetic antioxidants in treating these diseases. It has been proved experimentally that treating with enzymes-antioxidants (catalase and syperoxide dismutase) allowed to lower the number of pancreatic tissue derangements, changes in biochemical indices and extrapancreatic complications because of acute pancreatitis induced by natrium taurocholiate in rats (16).

The literature data concerning the oxidative stress of the organisms ill with digestive tract diseases are contradictory. Therefore the aim of the present work was to assess the peroxidative-antioxidative status of the organism in the cases of gastric ulcer, gastritis, duodenitis and ulcerative colitis, searching for new diagnostic criteria.

METHODS

The peroxidative status of test subjects was evaluated by the dienic conjugates (DC) and malondialdehyde (MDA) concentration in blood serum. For quantitative evaluation of lipid peroxides, their transformation into a coloured compound with thiobarbituric acid was applied (17). MDA, the final product of lipid peroxidation, reacts with thiobarbituric acid to afford a coloured complex which is characterised by absorption maximum at a wavelength of 532 nm. To establish the DC concentration in blood, we measured the lipid extract absorption in ultraviolet region at a wavelength of 232–234 nm (18). The results were expressed in nmol/ml.

The status of the antioxidative system was analyzed by measuring catalase activity. Catalase belongs to the enzymes that preserve their high activity for a long time without hardly demanding any activation energy; the reaction rate is limited by only the velocity of substrate diffusion into the active centre.

The core of the catalase determination principle is that we measure a time-related decrease of hydrogen peroxide content; hydrogen peroxide forms a stable coloured complex with ammonium molybdate. The intensity of the colour was measured at a wavelength of 410 nm. Enzyme activity is expressed in nmol/l/min (19).

RESULTS AND DISCUSSION

We have analyzed the LP relation with some digestive tract diseases. For this purpose, we have investigated 23 patients with gastric ulcer, 10 patients with gastritis, 10 with duodenitis, 10 subjects ill with ulcerative colitis. The control group consisted of 28 healthy subjects (blood donors).

When the status of the organism is normal, free-radical LP peroxidation products are formed and accumulate in the membranes, supporting their structure and function. The LP content is regulated by the ratio of the peroxidative and antioxidative processes. The intensity of peroxidative processes is enhanced by unsaturated fatty acids, active oxygen and the activity of the oxidative enzymes. Thus, on the one hand, lipid peroxides are constantly produced, but, on the other hand, they are constantly destroyed as a result of other processes. When the physiological conditions in the organism are normal, it contains a certain amount of lipid peroxides acccumulated in the membranes, providing for their normal physiological functioning.

In ulcer patients, LP processes in blood serum, particularly in the gastroduodenal mucous membrane, are intensified; therefore the membranes undergo deformation and become more permeable for hydrogen ions. The increased concentration of hydrogen ions evokes enzyme inhibition, catabolic processes in the cell and cell death (6).

Investigation of 23 ulcer patients showed that the MDA concentration in their blood serum was 1.6 times higher (6.01 ± 1.67 *versus* 3.44 ± 0.66 nmol/ml), and catalase activity was 1.3 times lower (35.63 ± 4.31 *versus* 45.33 ± 3.13 nmol/l/min) compared with the control.

Literature data also confirm highly intensified LP processes in gastric ulcer patients (20). In their blood plasma, the concentration of dienic conjugates and MDA was found to be two times and of Schiff bases 1.5 times higher. Such changes occur during suppression of the antioxidative system. This is also confirmed by a decrease of superoxidismutase (1.2 times) and ceruloplasmin (2 times) activity (7). In ulcer pathogenesis, an important role belongs also to hydroxyl ions (OH) which emerge under conditions of oxidative stress (MDA concentration increases, glutathione content and peroxidase activity decrease) [21]. Data obtained by some authors show that in ulcer patients the antioxidant concentration in their blood serum and mucous membrane is significantly lower than in control or in subjects ill with gastritis. Free radicals play an important role in gastroduodenal mucous membrane inflammation and in gastric cancer (22).

In our study, in gastritis patients MDA concentration was higher than in the control (5.82 ± 0.62 *versus* 3.44 ± 0.66 nmol/ml), and catalase activity was lowered to 31.77 ± 2.44 nmol/l/min. These indices did not differ much from those of ulcer patients. In duodenitis patients, the blood serum concentration of dienic conjugates, the primary index

of LP, levelled with that of control (5.68 ± ± 1.61 nmol/ml). With the progress of inflammatory process, MDA concentration increased 1.4 times (4.89 ± 1.61 *versus* 3.44 ± 0.66 nmol/ml), and the activity of the antioxidative enzyme catalase was 1.2 times as high (45.65 ± 3.83 nmol/l/min) as in ulcer patients. These data are in agreement with literature data.

Murine studies of duodenitis revealed an important role of free radicals. MDA concentration was found to be significantly higher, the antioxidative system much weaker; mieloperoxidase in the duodenic mucous membrane enhanced the accumulation of polymorphonuclear leucocytes (23).

Formation of free radicals is an important pathogenic factor in inflammatory intestinal diseases such as ulcerous colitis, Croh's disease. Changes in LP concentration measured by MDA and DC show the intensity of disease (17). In murine experimental colitis, a tight relation between oxidative stress and the antioxidative system of the organism has been revealed.

Reactive oxygen (oxygen free radicals) which causes oxidative stress is associated with inflammatory intestinal diseases (25). Free-radical oxygen conjugates have an influence on the development of ulcerative colitis. Free radicals damage the cell membranes and phagocyte functions (26). In the studies of the LP marker MDA and the activity of the antioxidative enzyme catalase in ulcerous colitis patients (n = 10), MDA concentration was found to be lower (2.85 ± 0.43 nmol/ml) and catalase activity higher (50.65 ± 4.36 nmol/l/min) than in healthy controls. These findings indicate a lowered activity of LP processes and an enhanced activity of the antioxidative system. Literature sources ascribe a significant role to LP in the pathogenesis of Croh's disease and ulcerative colitis (27). The activation of the inflamed cells and the hyperproduction of free radicals exert a considerable effect on the circulating products of LP. Hypercholesterolemia, higher MDA levels and a lowered retinol concentration have been stated. These data contradict our results. Besides, there are data allowing to question the effect of oxidative stress on the development of intestinal diseases, ulcerative colitis and Croh's disease (28, 31). In patients with inflammatory intestinal diseases, the parameters of blood serum antioxidants undergo changes, and the total potential of free radicals, depending on the severity of disease, becomes lower (29).

Our data correlate with the results of other authors where gastric cancer is concerned. Some works show that LP is intensified and the antioxidative system becomes weaker [30, 31]. In other cases it has been stated that in various forms of cancer and with the growth and progressing of tumour the cholesterol and MDA content decreases. A multivariate analysis and studies into the correlation between lipid concentration and tumorous cell growth have shown that the metastases are related with a low vitamin E and cholesterol ratio and low MDA concentration (10, 15). Thus, derangements of LP regulation and exhaustion of the antioxidative system of the organism are closely related with a number of digestive tract diseases.

Changes in the LP indices – concentrations of the secondary peroxidation metabolites, dienic conjugates and malondialdehyde, as well as variations of the antioxidative enzyme, catalase in blood serum have been studied. The data obtained have revealed higher concentrations of MDA and the lowered activity of catalase in ulcer and gastritis patients, depending on the character and severity of the disease. Increase in MDA concentration is related with a lower catalase activity. This means an intensified peroxidation and a suppression of the antioxidative process.

CONCLUSIONS

1. In ulcer and gastritic patients, lipid peroxidation has been found to be 1.6 times more intensive and the activity of the enzyme catalase 1.3 times lower than in healthy controls.

2. No essential disease-related difference has been found among the indices of the patients ill with ulcerous disease and gastritis.

3. In duodenitis patients, compared with control, the concentration of dienic conjugates did not change, MDA concentration was 1.5 times higher, and catalase was 1.2 times more active than in ulcer patients.

4. In ulcerative colitis patients, the intensity of LP decreases and of catalase increases. The antioxidant system of the organism seems to be potent enough so that the enzymes of this system can neutralize the formation of free oxygen radicals.

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OKSIDACINIO STRESO VAIDMUO SERGANT VIR□KINIMO TRAKTO LIGOMIS

S a n t r a u k a

Darbo tikslas – įvertinti organizmo peroksidacinės-antioksidacinės būklės pokyčius sergant opalige, gastritu, duodenitu ir opiniu kolitu. Buvo ištirti 43 ligoniai ir 28 sveiki žmonės. Nustatyta malondialdehido koncentracija ir katalazės aktyvumas ligonių kraujo serume, palyginta su rodiklių reikšmėmis sveikų asmenų serume. Sergant opalige, ir gastritu, lipidų peroksidacija suintensyvėja, katalazės aktyvumas sumažėja, palyginti su kontrole sveikų žmonių kraujo serume. Sergant opiniu kolitu, LP intensyvumas mažėja, o katalazės didėja. Matyt, antioksidacinė organizmo sistema yra labia pajėgi, todėl šios sistemos fermentai gali neutralizuoti laisvųjų deguonies radikalų susidarymą.