BIOCHEMICAL MODIFICATIONS OF GASTRIC MUCOSA IN OXIDATIV STRESS

MODIFICĂRI BIOCHIMICE ALE MUCOASEI GASTRICE ÎN STRESUL OXIDATIV

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This study aims to explain the participation of ROS in chronic gastric mucosal damage by drinking water with pH 4 and by undergoing 30 minutes for three times per day of water immersion. After four weeks the animals which were exposed to two damaging factors were sacrificed and gastric mucosa was collected for analyzing lipid peroxidation and superoxide dismutase activity. The levels of MDA and 4-HNE used as indicators of lipid peroxidation, increased from 5.85 ± 0.04 nmol/g to 12.25±0.95 nmol/g for acid group and from 5.85 ± 0.04 nmol/g to14.06 ± 1.20 nmol/g for water immersion group. In the acid group the level of total glutathione decreased to 200.10±19.10 mg/100g and 145.56±13.85 mg/100g reduced glutathione. In water immersion group the level of total glutathione decreased to 180.70 ±16.82 mg/100g and 130.60±10.64 mg/100g reduced glutathione. In acid group Superoxide dismutase decreased to 255.18 ± 22.84 U/g and in water immersion group decreased to 215.73 ± 20.60 U/g.

Keywords: gastric mucosa, superoxide dismutase, malondialdehyde, stress

Introduction

The mucosal barrier is composed by epithelial cells with tight junctions and superimposed layer of mucus. The aim of this barrier is to protect the mucosa against damage of deeper structures by hydrogen ions (H+) and other noxious substances originating from the gastric lumen (Konturek, 1997). The endogenous prostaglandins (PGs) play an important role in the maintenance of mucosal integrity, which includes continuous secretion of bicarbonate anions (HCO3-) and a mucus production in the stomach and duodenum (Brzozowski et al., 2000). The imbalance between gastrotoxic agents and protective mechanisms results in an acute inflammation. This acute inflammation is accompanied by neutrophils infiltration of gastric mucosa. Neutrophils produce superoxide radical anion (O2•-), which belongs to group of reactive oxygen species (ROS). Superoxide radical anion reacts with cellular lipids, leading to the formation of lipid peroxides, that are metabolized to malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). The
body has several enzymatic systems, which scavenges ROS and prevents their destructive action. The major antioxidative enzyme is superoxide dismutase (SOD). Three types of superoxide dismutase (SOD) can be distinguished: cytoplasmatic, mitochondrial and extracellular. SOD catalyzes the dismutation of superoxide radical anion (O2•-) into less noxious hydrogen peroxide (H2O2), that is further degraded by catalase or glutathione peroxidase. Catalase is an enzyme which accelerates degradation of H2O2 into water and oxygen (Halliwell, 1990). The second pathway of H2O2 metabolism depends on the activity of glutathione peroxidase (GPx) and cooperating glutathione reductase. The reduction of H2O2 into water by GPx is accompanied by the conversion of glutathione from reduced form (GSH) into oxidized form (GSSG).

The aim of our present investigations is to demonstrate the participation of ROS in gastric mucosal damage by various irritants.

Materials and Methods

Twenty four Wister white rats, weighing 120±20 g from Biobaza Cantacuzino were used. They were housed in plastic cages, 8/cages, in identical conditions of temperature (18°-20°C) and humidity (40-60°C). They were divided into three groups: a control group, which drank only water (pH 6.5), an "acid" group, which drank syrup water with acetic acid (pH 4), and a "water immersion stress" group. In 3rd group of animals underwent 30 minutes for three times per day of water immersion restraint stress in temperature 20°C. After four weeks they were sacrificed and gastric mucosa was collected and homogenized for analyzing lipid peroxidation (MDA and 4-HNE tissue concentration) with colorimetric assay (Bioxytech LPO-586, Oxis, Portland, USA). Results were expressed as nanomol per gram of tissue (nmol/g) (Esterbauer et al., 1991). To determine activity of superoxide dismutase (SOD), we used the colorimetric assay (Bioxytech, SOD-525, Oxis, Portland, USA). Results were expressed as units per gram of tissue (U/g) (Archibald, 1990). The concentration of total and reduced glutatione was found out applying DTNB (5,5′ Dithio-bis-2 nitrobenzoic acid), method forming TNB (5THIO-2 nitrobenzoat) whose absorption was measured at λ 412 nm (Harmut, 1990). The results were expressed in mg at 100 g gastric tissue.

Results were expressed as means ±SEM and were statistical analysis using „t“Test. Differences with p<0.05 were considered as significant.

Results and Discussion

Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) in tissue are accepted as major products of lipid peroxidation. They are considered indicators of mucosa injuring by ROS. Concentration of MDA and 4-HNE in intact mucosa was at very low level, near to the analytical limit of detection, averaging 5.85 ± 0.04 nmol/g of tissue. After the administration of syrup water with acetic acid (pH 4), the levels of MDA and 4-HNE increased to 12.25±0.95 nmol/g and in case of water
restraint stress, the level of lipid peroxides metabolites increased to $14.06 \pm 1.20$ nmol/g. These outcomes, in all investigated groups, were significantly higher, as compared with the values obtained in the intact mucosa (figure 1).

![Figure 1](image1.png)

**Figure 1.** Concentration of MDA and 4-HNE (nmol/g) in the gastric mucosa in rats exposed to application of acetic acid (pH 4) and 30 min. for three times per day of water immersion (temp. 20°C) compared with the control group values. Results are mean ± SEM.

The total and reduced glutathione were analyzed for three groups. In 1st group the total glutathione was $230.20 \pm 20.12$ mg/100g and $170.32 \pm 97.66$ mg/100g reduced glutathione. In 2nd group the level of total glutathione decreased to $200.10 \pm 19.10$ mg/100g and $145.56 \pm 13.85$ mg/100g reduced glutathione. In 3rd group by water immersion stress the level of total glutathione decreased to $180.70 \pm 16.82$ mg/100g and $130.60 \pm 10.64$ mg/100g reduced glutathione (figure 2).

![Figure 2](image2.png)

**Figure 2.** Total and reduced glutathione expressed as mg/100 g of fresh gastric mucosa in rats exposed to application of acetic acid (pH 4) and 30 minutes for three times per day of water immersion (temp. 20°C) compared with the control group values. Results are mean ± SEM.
Enzymatic activity of superoxide dismutase (SOD) is a measure of antioxidative properties of cells. The activity of SOD in intact gastric mucosa reached high level, 340.30 ± 28.77 U/g of tissue. In 2nd group significant decrease resulted to 255.18 ± 22.84 U/g and in 3rd group an insignificant decrease resulted to 215.73 ± 20.60 U/g (figure 3).

Figure 3. SOD activity (U/g) in the gastric mucosa in rats exposed to application of acetic acid (pH 4) and 30 minutes for three times per day of water immersion (temp. 20°C) compared with the control group values. Results are mean ± SEM.

These values correlated with those of MDA concentration, namely the decrease of SOD activity induces favourising conditions for cell membrane lipoperoxidase. Consequently, under conditions of moderate oxidative stress, SOD activity increases demonstrating the effort of organisms to balance the oxidative effect, which enhances lipidperoxidative processes. During the process of prolonged or intensive oxidative stress SOD activity increases much owing to the enzyme inactivity.

Previous studies focused on the participation of ROS in pathogenesis of gastric diseases. This disease is more common than we think but they can be difficult to confirm. In digestive system investigations on ROS of pancreas liver and small intestine predominantly concerned (Simovic, 1997). Little information is available regarding the formation of ROS into esophagus and gastric mucosa, exposed to various damaging factors.

Erin et al. (2000) attempted to explain the mechanism of radical production. He examined pathomechanisms of gastric mucosa damage, resulting from thermal stress. Animals in Erin’s model, underwent thermal stress, in temperature 6°C, during 4 h. Erin et al. failed to observe any significant changes in MDA level in stressed stomach. In our investigations we applied different approach, animals underwent 30 minutes for three times per day of water immersion restraint stress.
(WRS) in temperature 20°C. Under these stress conditions, a significant increase of MDA level after WRS, accompanied by decrease of enzymatic activity of antioxidative enzyme-superoxide dismutase (SOD) were observed.

Previous research on metabolism of ROS in gastric mucosa focused on the effects of Helicobacter pylori infection. Davies at al., (1994) showed that such infection of human gastric mucosa resulted in an increase of ROS production, measured by chemilumimetry, as compared with healthy mucosa. In our investigations we confirmed that exposed gastric mucosa to oxidative stress, induced by drinking syrup water with acetic acid (pH 4), or water immersion in temperature 20°C undergoing 30 minutes for three times per day leads to the generation of lipid peroxides, as expressed by an increase of tissue level of MDA accompanied by impairment of antioxidative defense mechanisms, such as decrement in SOD activity. Experiments carried out till now focused on measurement of MDA level, or its derivatives, in rat’s liver after ethanol application (Chen 2000).

Our experiments indicate that intensification of ROS production results in lipid peroxidation, expressed by tissue increment of MDA and 4-HNE levels. These phenomena are accompanied by impairment of antioxidative properties of cells, what is supported by our finding of the decrease of SOD activity in gastric mucosa.

**Conclusions**

1. MDA concentration increased significantly for the group under water immersion and it is negatively correlated with SOD activity obtained from gastric mucosa.
2. The decrease of SOD activity favourised ROS attack at the level of gastric mucosa.

**References**


