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OXIDATIVE STRESS IN RATS GASTRIC TISSUES WHEN SIMULATING GASTROPATHY AT LACK AND EXCESS OF MELATONIN

Abstract. *The study has shown that different models of effects on the rats stomach, such as immobilization stress and chemical gastritis, against the background of a short lack and excess of melatonin cause different reactions of the prooxidant-antioxidant system. At the immobilization stress melatonin has gastroprotective effect, and at the simulated chemical gastritis it enhances the oxidative stress.*

Key words: *oxidative stress, prooxidant-antioxidant system, stomach, gastropathy, melatonin.*

Introduction. For the first time the term "oxidative stress" appeared in the 80s of the twentieth century when the German scientist Helmut Sies gave the following definition: "This is an imbalance of prooxidant-antioxidant system in favor of the prooxidants" [1]. The key concepts in this definition are the prooxidant and antioxidant.

The most active prooxidants in living systems are free radicals and ion-radicals, which contain unpaired electrons in atoms of oxygen, nitrogen, sulfur, chlorine: primarily they are superoxide anion ($O2^{\cdot -}$) that when interacting with other compounds turns into hydrogen peroxide (H2O2), which is not a radical, and very reactive hydroxyl radical ($HO^{\cdot}$), singlet oxygen (O21) and peroxynitrite ($ONOO^{-}$). All these forms of reduced oxygen ($O2^{\cdot -}$, H2O2, $NO^{\cdot}$) have a higher reactivity than oxygen molecule, so they were unifying called "reactive oxygen species" (ROS) [2,3].

The influence of oxygen and ROS leads to the formation of a number of organic radicals: R•, RO•, ROO•. A free radical actively react with neighboring molecules, thus forming a new radical, which causes a chain reaction. The stable level of free radical peroxidation (FRPO) in the body is maintained by the activity of the enzyme and non-enzymatic antioxidant systems (AOS).

AOS includes both non-enzymatic antioxidants: tocopherol, vitamin A, carotenoids, ubiquinone, ascorbic acid, thiol compounds, selenium derivatives, transferrin, lactoferrin, albumin, and enzymes: superoxide dismutase (superoxide: superoxide oxidoreductase, EC 1.15.1.1), catalase (hydrogen peroxide: hydrogen peroxide oxidoreductase, EC (1.11.1.6), glutathione peroxidase (glutathione, hydrogen peroxide oxidoreductase, EC 1.11.1.9) and glutathione reductase (EC 1.6.4.2), ceruloplasmin (iron II: oxygen oxidoreductase, EC 1.16.3.1) (Tsebrzhinskyi O.I., 2001, Baraboi V.A.,2006).

A number of factors leads to an imbalance of the prooxidant-antioxidant system. They are a stress, action of chemical and physical factors, the impact of xenobiotics, etc., leading to increased activity of free radical processes and consequently to increased concentrations of primary and secondary peroxidation products.

Hans Selye (1936) noted that the effect of numerous negative factors on the body, regardless of their nature and properties, have a standard response that is manifested as morphological triad: hypertrophy of the adrenal cortex; involution of thymus-lymphatic system (atrophy of the thymus and lymph nodes); formation of erosions and ulcers in the gastrointestinal tract.

At the stress the hypothalamic-pituitary-adrenal and sympathoadrenal systems activate. Their hormones influence the increased secretion of hydrochloric acid, pepsin, lead to

hypertonus of stomach, ischemia of gastric vessels. This causes intragastric proteolysis and damage to the mucosa [4,5,6]. Besides stress, including immobilization one, leads to increased FRPO, and as a result - to oxidative violation of tissue integrity, which causes gastroduodenal erosions and ulcers [7,8]. The imbalance in favor of FRPO with increasing concentrations of peroxidation products is the oxidative stress, which eventually leads to violation of tissues integrity. Therefore, there is a new deeper definition of "oxidative stress" in the scientific literature: "This is a temporary or permanent increase in level of ROS (compared to the stationary level), which violate the cellular integrity and metabolism, including processes with the participation of ROS, leading to oxidative modification of cellular components, which at a significant imbalance of PAS can lead to cell death by apoptosis or necrosis" (Lushchak, 2015).

In modern studies the following question should be focused on: "What can correct acute and chronic oxidative stress?" To prevent FRPO and correct pathologies caused by free radicals a range of antioxidants is used in modern medical practice.

Melatonin is such a stress protector, stimulator of the immune system, gero- and cancer protector, antioxidant (AO). It can positively affect the digestive tract and have anti ulcerogenic effect [9,10,11,12]. Its antioxidant action is due to its ability to neutralize free radicals and act as an indirect antioxidant activating antioxidant enzymes: superoxide dismutase (SOD), glutathione peroxidase (GPO) [13].

Objective: to determine changes of PAS when simulating gastropathy against the background of lack and excess of melatonin and to find out whether melatonin have the gastroprotective effect at various types of gastropathy.

Materials and methods. Study was performed on 90 Wistar rats. Keeping of animals and experiments were carried out due to the requirements of “European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes” (Strasbourg, 1986) and “General Ethical Principles of Animals Experiments”, ratified on the First National Congress of bioethics (Kyiv, 2001).

Hypomelatoninemia (lack of melatonin) was simulated by the method described by V.M. Huraliuk (2008) [14] by non-stop lighting during 10 days. Since melatonin is synthesized in the epiphysis only in darkness, we supposed that the simulation partly reproduces hypomelatoninemia, considering that there is non-stop synthesis of the hormone by APUD system. It is known that power of light of 0.0005 mV/cm^2 is enough for white rats to reduce the amount of synthesized melatonin [15].

Maximum dose of melatonin single introduction, according to the literature [16, 17], is 5 mg/kg of body weight. In the experiment hypermelatoninemia was simulated by the introduction of melatonin at a dose of 1 mg/kg per day during 10 days.

Two types gastropathy were simulated in experiments: Selye stress ulcer and formation of erosions against the background of introduction of acetic acid. 24 hours before the simulation of gastropathy animals were not fed, but they had free access to water, because the starvation due to the activation of anaerobic glycolysis results in the reduction of protective factors in the gastric mucosa. The stress ulcer was simulated according to Selye by immobilization of the animals in tight boxes for 3 hours. Neuro-humoral factor plays a leading role in the mechanism of genesis of the simulated "stress ulcer", that is why this model is the most appropriate one among quick "acute" experimental ulcers (Zavodskaia I.S., Moreva E.V., 1981). The second type of gastropathy was simulated by the one-time introduction of 10% solution of acetic acid per os through a probe under light anesthesia. Gastropathy was simulated on the ninth day of the experiment, and on the tenth day the euthanasia was performed by bleeding with hexanal anesthesia due to the requirements of bioethics. The stomachs were used for further studies.

After macroscopic study the level of erosive and ulcerative damage to gastric mucosa was evaluated. Number of ulcers in gastric mucosa,

the average number of destructions per animal in the group, the percentage of animals with ulcers in the group (Hadzhai Ya.I. et al., 1962) were calculated, and the Pauls index (Pauls F., Wick A.M., Mac Key E.M. et al., 1947; Dobriakov Yu.I., 1978) [18, 19, 20] was calculated as follows:

$$\frac{n_{\_\text{the average number of destructio1animal}}}{100} \times \%animals\_with\_ulcer$$

Also the antiulcer activity – AUA was calculated (the ratio of the Pauls index of control group to the one of experimental group).

Concentration of primary (diene conjugates (DC) by the method of Stalna I.D.) and secondary (thiobarbituric acid (TBA)-active products by the method of Stalna I.D., Haryshvyly T.G.) products of free peroxidation in the stomach homogenate was determined. Also activity of catalase by the method of Koroliuk M.A., Ivanova L.I., activity of glutathione peroxidase (GPO) by the method of Pakhomova V.A., activity of superoxide dismutase (SOD) by the method of Chevary S., I. Chaba I., and general proteolytic activity (GPA) by the method of Kunitz in stomach homogenate were determined.

The following methods of statistical analysis were used: checking of normality of distribution using the Shapiro-Wilk criterion, the reliability of the difference between the averages of each of biochemical parameters in the study groups was determined by Student t-test. Statistical analysis was carried out using a computer program Microsoft Office Excel 2007.

Results and discussions. As the study has shown, the content of primary lipid peroxidation products and TBA-active products when simulating immobilization stress against the background of the 10-day and normo-, hypo- and hypermelatoninemia changes, functioning of enzyme antioxidant protection changes too (Table 1).

There is an acute oxidative stress in animals in stressful conditions, which is characterized by

Table 1.

Changes of some parameters of PAS when simulating immobilization stress against the background of lack and excess of melatonin, (M±m)

Parameters	Experimental conditions					
	Natural lighting Normomelatoninemia		Permanent lighting Hypomelatoninemia		Natural lighting+ melatonin introduction Hypermelatoninemia	
	Without stress (Intact) (n=8)	At stress (n=8)	Without stress (n=8)	At stress (n=8)	Without stress (n=8)	At stress (n=8)
DC mmol/kg	6.438± 0.365	11.01± 0.513***	6.75± 0.571	9.26± 0.371*** ▪	4.431± 0.589 *	8.699± 0.498** ▪▪
TBA-active products, µmol/kg	7.821± 0.26	19.81± 2.68**	8.014± 0.34	13.51± 1.66**	9.23± 1.54	13.4± 2.35*
Activity of SOD, st.units/g	0.285± 0.05	0.214± 0.05	0.143± 0.033*	0.182± 0.016	0.219± 0.027	0.168± 0.031
Activity of catalase, mkat/kg	2.559± 0.074	2.09± 0.09**	2.113± 0.106 **	2.116± 0.076***	2.895± 0.131*	2.195± 0.042***
Activity of GPO mkat/kg	5.46± 0.308	5.07± 0.293	4.64± 0.312	4.93± 0.442	4.11± 0.302**	4.41± 0.276*

*Note: *, **, *** - the difference between the samples means is statistically significant with ρ≤0.05, ρ≤0.01, ρ≤0.001 respectively compared to the intact group; ▪, ▪▪, ▪▪▪ the difference between the samples means is statistically significant with ρ≤0.05, ρ≤0.01, ρ≤0.001 respectively compared to the control stress.*

sharp changes in parameters of PAS: the concentration of diene conjugates in the gastric tissues homogenates increases in 1.7 times compared to the intact group (by 71,02% (ρ = 0.001)); the concentration of TBA-active products in rat gastric tissues homogenate increases in 2.5 times compared to the intact group (ρ = 0.001); there is a decrease in enzyme antioxidant system activity - SOD activity decreases slightly (by 24.9%), catalase activity decreases slightly (by 18.3% (ρ≤0.01)), GPO activity decreases slightly (by 7%).

Also there is an acute oxidative stress at immobilization stress against the background of lack of melatonin: DC concentration in the gastric tissues homogenates increases compared to the intact group (by 43.8% (ρ = 0.001), but is lower than at the stress control (by 15.9% (ρ≤0.05)); the concentration of TBA-active products in rat gastric tissues homogenate increases in 1.7 times compared to the intact group (ρ≤0.01) but is slightly lower than at the stress control; also there is a decrease in enzyme antioxidant system activity - SOD activity decreases slightly (by 36.1%), catalase activity decreases slightly (by 17.3% (ρ=0.001)), GPO activity decreases slightly (by 9.7%) compared to the intact group. The acute oxidative stress also occurs at the immobilization stress against the background of introduction of melatonin per os at a dose of 1 mg/kg: DC level at hypermelatoninemia increases compared to the intact group (by 31.2% (ρ≤0.01)), but is lower by 21% compared to the stress control group (ρ = 0.01) and slightly lower than at stress with hypomelatoninemia; the concentration of TBA-active products in gastric tissues homogenate increases in 1.7 times (71% (ρ≤0.05)) but is slightly lower than at the stress control (32.4%); SOD activity decreases slightly compared to the intact group (41.1%) and almost does not differ from the activity at the stress and at the stress with hypomelatoninemia; catalase activity decreases (by 14.2% (ρ = 0.001)), but also does not differ from the activity at the stress and at the stress with hypomelatoninemia; GPO activity also decreases (by 19.2% (ρ≤0.05)) compared to the intact group and is lower than at the stress control (13%).

The study shows the increase in the severity of ulcerative process in the rats stomach at simulated immobilization stress: the frequency of damages increases, the average number of destructions per animal increases, the average degree of ulcer in the group increases, the Pauls

Table 2.

Morphological changes in gastric mucosa of white rats at simulated immobilization stress on the 10th day of the normo-, hypo- and hypermelatoninemia, (M±m)

Parameters	Experimental conditions					
	Natural lighting Normomelatoninemia		Permanent lighting Hypomelatoninemia		Natural lighting+ melatonin introduction Hypermelatoninemia	
	Without stress (Intact) (n=8)	At stress (n=8)	Without stress (n=8)	At stress (n=8)	Without stress (n=8)	At stress (n=8)
Frequency of damages, %	12.5	87.5***	0	100***	25**	62.5**
Average number of destructions per rat	0.25	9.63***	0	13.25***	0.5**	3.38**
Average level of ulcer (LU) in the group	0.125	1.5***	0	2.125***	0.375**	1***
The Pauls index (PI)	0.031	8.42***	0	13.25***	0.125***	2.11***

*Note: *, **,*** - the difference between the samples means is statistically significant with ρ≤0.05, ρ≤0.01, ρ≤0.001 respectively compared to the intact group*

index increases (Table 2), and there is a significant worsening in all the parameters at immobilization stress against the background of hypomelatoninemia.

Our calculations of antiulcer activity (the ratio of the Pauls index of control group to the one of experimental group with simulated immobilization stress against the background of hypermelatoninemia) indicate the gastroprotective activity of melatonin.

Analyzing the data, it can be noted that prophylactic introduction of melatonin have not prevented the emergence of acute oxidative stress in this model of gastropathy but immobilization stress caused less damage to the gastric mucosa (GM).

But as it is shown by the study, at another model of gastropathy, namely the chemical gastritis, the parameters of prooxidant system and antioxidant enzymes changes in different way (Table 3).

At the simulated chemical gastritis there is no

Table 3.

Changes of some parameters of PAS when simulating chemical gastritis against the background of lack and excess of melatonin, (M±m)

Parameters	Experimental conditions					
	Natural lighting Normomelatoninemia		Permanent lighting Hypomelatoninemia		Natural lighting+ melatonin introduction Hypermelatoninemia	
	Without effect on GM (n=7)	At the simulated chemical gastritis (n=7)	Without effect on GM (n=7)	At the simulated chemical gastritis (n=7)	Without effect on GM (n=7)	At the simulated chemical gastritis (n=7)
DC mmol/kg	6,305 ± 0,102	4,617 ± 0,206**	7,812 ± 0,314***	14,42 ± 1,311***■■■	7,759 ± 0,177***■■■	10,33 ± 0,363***■■■
TBA-active products, µmol/kg	5,769 ± 0,228	7,359 ± 0,422**	19,5 ± 1,269***■■■	12,82 ± 0,759***■■■	95,88 ± 0,824***■■■	100,6 ± 1,063***■■■
Activity of SOD, st.units/g	0,644 ± 0,032	0,373 ± 0,096*	0,56 ± 0,065■■	0,264 ± 0,048***	1,161 ± 0,026***■■■	1,147 ± 0,037***■■■
Activity of catalase, mkat/kg	2,1 ± 0,088	2,166 ± 0,084	1,542 ± 0,205*■■	1,424 ± 0,164**■■■	1,928 ± 0,016	1,788 ± 0,094*■■
Activity of GPO mkat/kg	5,69 ± 0,172	7,88 ± 0,251***	7,32 ± 0,263***	8,14 ± 0,09***	7,31 ± 0,088***	7,98 ± 0,145***

*Note: *, **,*** - the difference between the samples means is statistically significant with ρ≤0.05, ρ≤0.01, ρ≤0.001 respectively compared to the intact group; ■■, ■■■ the difference between the samples means is statistically significant with ρ≤0.01, ρ≤0.001 respectively compared to the group with chemical gastritis*

acute oxidative stress in animals: the concentration of diene conjugates in the gastric tissues homogenates decreases slightly compared to the intact group (by 26.8% (ρ = 0.01)); the concentration of TBA-active products in the gastric tissues homogenates of rats increases compared to the intact group (by 27.6% (ρ = 0.01)); SOD activity decreases in 1.7 times (ρ≤0.05); catalase activity does not change; and GPO activity increases (by 38.5% (ρ = 0.001)).

There is an acute oxidative stress at simulated chemical gastritis against the background of 10-day hypomelatoninemia: the

concentration of diene conjugates in the gastric tissues homogenates increases in 2.3 times (ρ = 0.001); the concentration of TBA-active products in the gastric tissues homogenates of rats increases in 2.2 times compared to the intact group (ρ = 0.001); there is a decrease in enzyme antioxidant system activity - SOD activity decreases compared to the intact group in 2.4 times (ρ = 0.001) and is lower than in the group with chemical gastritis (by 29.2%); catalase activity also decreases compared to the intact group (by 32.2% (ρ = 0.01)) and is also lower than in the group with chemical gastritis (by 34.3% (ρ = 0.001)); and only GPO activity increases compared with the intact group (by 43.1% (ρ = 0.001)), and almost does not differ from the group with the simulated chemical gastritis.

At the simulated chemical gastritis against the background of 10-day hypermelatoninemia contents of primary and secondary products are also high: thus DC concentration increases compared to the intact group (by 63.8% (ρ = 0.001)), but decreases compared to the group with the simulated chemical gastritis at the hypomelatoninemia (by 28.4% (ρ≤0.05)); the concentration of TBA-active products in the gastric tissues homogenates of rats increases in 17.4 times (ρ = 0.001) and increases in 7.9 times (ρ = 0.001) compared to the group with the simulated chemical gastritis at the hypomelatoninemia. As for the antioxidant system, there is an increased activity of SOD compared to the intact group in 1.8 times (ρ = 0.001), compared to the group with the chemical gastritis - in 3 times (ρ = 0.001), compared to the group with the chemical gastritis at the hypomelatoninemia - in 4.4 times (ρ = 0.001). The activity of catalase is lower than in the intact group (by 14.9% (ρ≤0.05)) and is slightly higher than in the group with the chemical gastritis at the hypomelatoninemia (by 16%). GPO activity is higher than in the intact group (by 40.3% (ρ = 0.001)), and does not differ from the activity of the group with the chemical gastritis and of the group with the chemical gastritis at the hypomelatoninemia.

As it can be seen from Table 4 at the simulated chemical gastritis there is an increase in the severity of ulcerative process in the stomach of rats compared to the intact group: the frequency of damages increases, the average amount of destructions per animal increases, the average level of destructions in

Table 4.

Morphological changes in gastric mucosa of white rats at simulated chemical gastritis on the 10th day of the normo-, hypo- and hypermelatoninemia, (M±m)

Parameters	Experimental conditions					
	Natural lighting Normomelatoninemia		Permanent lighting Hypomelatoninemia		Natural lighting+ melatonin introduction Hypermelatoninemia	
	Without effect on GM (n=7)	At the simulated chemical gastritis (n=7)	Without effect on GM (n=7)	At the simulated chemical gastritis (n=7)	Without effect on GM (n=7)	At the simulated chemical gastritis (n=7)
Frequency of damages, %	0	100***	100***	100***	57.14***	100***
Average number of destructions per rat	0	3.43***	5.43***	7***	3***	13***
Average level of ulcers (LU) in group	0	1.57***	1.71***	2.29***	0.86***	2.57***
The Pauls index (PI)	0	3.43***	5.43***	7***	1.71***	13***

*Note: *, **,*** - the difference between the samples means is statistically significant with ρ≤0.05, ρ≤0.01, ρ≤0.001 respectively compared to the intact group*

the group increases, the Pauls index increases. At the lack of melatonin and simulated chemical gastritis all the parameters worse, but at the hypermelatoninemia and chemical gastritis all the parameters worse even more compared to the group with the chemical gastritis: the average amount of destructions increases in 3.8 times, the average degree of ulcer increases in 1.6 times, the Pauls index rises in 3.8 times.

Also the antiulcer activity of melatonin AUA was calculated (the ratio of the Pauls index of the group with the chemical gastritis to the one of the group with the chemical gastritis at the hypermelatoninemia): in this model of gastropathy melatonin does not reveal his activity, as (due to Pauls, 1947) AUA value should be higher than 2 units.

Conclusions: 1. There is an acute oxidative stress in the rat gastric tissues at the simulation of gastropathy by the immobilization stress.

2. There are no significant changes of parameters of PAS at the simulated chemical gastritis, which indicates the absence of the acute oxidative stress.

3. In the group with the simulated immobilization stress the introduction of melatonin at a dose of 1 mg/kg per day during 10 days has gastroprotective effect.

4. In the group with the simulated chemical gastritis the introduction of melatonin at a dose of 1 mg/kg per day during 10 days does not have gastroprotective effect, and the concentration of free radical peroxidation products is higher than in all other experimental groups, which indicates the presence of the acute oxidative stress.

5. Reactions of prooxidant-antioxidant system of rat stomach at various types of are different, which indicates there are different mechanisms of adaptation of the system to the action of negative factors.

Prospects for further research. It is reasonable to perform further study of the reactions of prooxidant-antioxidant system in the stomach at the oxidative stress against the background of the prolonged lack and excess of melatonin.

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