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STUDY OF THE ACTIVITY OF THE ANTIOXIDANT SYSTEM IN EXPERIMENTAL PERIODONTITIS

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ABSTRACT — The aim of this study was to evaluate the activity of the antioxidant system when creating a model of periodontitis in an experiment in rats. We made a model of periodontitis in rats (n = 10) by ligating the first two molars with a ligature. The control group consisted of intact rats (n = 10). In all animals, we determined the level of activity of catalase, superoxide dismutase and free hydroxyproline in blood plasma on the 3rd, 7th, 25th days. As a result, we revealed a significant decrease in the activity of enzymes of the antioxidant system in the main group compared with the control (p <0.001). Also, the rats of the main group had a negative correlation between the level of catalase (r = -0.84), and superoxide dismutase (r = -0.79) in relation to the parameters of free hydroxyproline.
CONCLUSION: Low antioxidant status in rats with periodontitis leads to destruction of collagen structures in periodontal tissues.

KEYWORDS — periodontitis, oxidative stress, periodontal diseases, catalase, superoxide dismutase, collagen.

INTRODUCTION

Periodontitis is a chronic inflammation of the tooth-supporting tissues [1]. Almost every adult faces periodontitis, at least once in a lifetime. Due to chronic inflammation in the periodontal tissues, neutrophils and lymphocytes are activated enabling synthesis of cytokines and prostaglandins [2]. Long-term chronic inflammation in tissues leads to an increase in reactive oxygen species (ROS), and an imbalance in the antioxidant system [3, 4]. The long-term chronic inflammation is known to facilitate destruction of tissues supporting teeth, including the gums, periodontal ligament, and alveolar bone [5]. Also, chronic periodontitis is the main cause of tooth loss, which significantly impairs quality of life in such patients. Therefore, assessment of oxidative processes, identification of the correlation between activity of the antioxidant system and destruction of collagen structures of periodontal tissues is an urgent issue of modern dentistry.

Aim:

To assess the activity of the antioxidant system when creating a model of experimental periodontitis in rats.

MATERIAL AND METHODS

The experiments were performed on 20 healthy outbred rats aged 12 to 16 weeks and weighing 180 to 225 g. All procedures with animals were performed according to the rules of the Guide for the care and use of laboratory animals. We randomly assigned the rats into two groups:

— control group (n = 10) — animals that were not manipulated

— the main group (n = 10) — animals for which a model of periodontitis was created.

We carried out all the manipulations in animals under general anesthesia (0.03 ml/m). To create a model of periodontitis, we applied a ligature (Vicryl 5.0) between the two molars, while we tried to suture the interdental papilla between the first and second molars of the upper jaw on the left. This ligature acted as an irritant to the gums and caused accumulation of bacterial plaque, followed by the development of periodontitis after 30 days.

Further, we evaluated the intensity of oxidative stress and investigated the key enzymes of the antioxidant system — catalase and superoxide dismutase in blood plasma. We determined the activity of the above enzymes in both groups of animals using kits for spectrophotometric analysis. We determined the degree of collagen destruction by the change in the level of free hydroxyproline (mg/l). Oxyproline is one of the essential amino acids in collagen. Consequently, fluctuations in the level of this indicator indicate the intensity of the degradation of collagen structures. We determined the oxyproline of blood serum by the colorimetric method.

The results were monitored after modeling periodontitis and removing the ligature on days 3, 7 and 25. During the observation, all animals were kept in the same conditions and were fed soft food.

Statistical processing was carried out with the calculation of arithmetic mean values (M) and their errors (m). The reliability of differences in the groups was calculated using the Mann-Whitney test. The differences were considered significant if p <0.05.

RESULTS

The indicators of the activity of the antioxidant system before the start of the study did not significantly differ between the main and control groups of animals. After creating a model of periodontitis, throughout the observation period, the indicators of catalase and superoxide dismutase activity in blood serum significantly differed between the groups (Table 1, 2).

Table 1. *Indicators of catalase activity (mmol / l) in blood plasma of animals of both groups*

Research days / Animal groups	Before the research	3rd day	7th day	25th day
Control group (n=10)	1,17±0,05	1,16±0,04	1,19±0,03	1,15±0,03
Main group (n=10)	1,15±0,02	0,51±0,02	0,44±0,04	0,40±0,03
p	p>0,05	p <0,001	p <0,001	p <0,001

Table 2. *Indicators of the level of superoxide dismutase (U / ml) in the blood plasma of animals of both groups*

Research days / Animal groups	Before the research	3rd day	7th day	25th day
Control group(n=10)	1,30±0,04	1,31±0,02	1,29±0,03	1,32±0,04
Main group (n=10)	1,31±0,03	0,45±0,04	0,48±0,05	0,52±0,06
p	p>0,05	p <0,001	p <0,001	p <0,001

The level of free hydroxyproline in rats of the main group was significantly higher than in the control (p <0.001), which indicates a high metabolic activity of collagen-containing structures of connective tissue in periodontitis (Fig. 1).

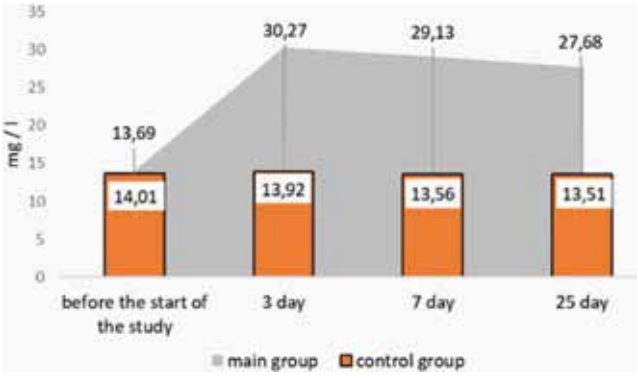

Fig. 1. *Dynamics of changes in the level of free hydroxyproline (mg/l) in animals of both groups*

At the end of the experiment, we found that we found a negative relationship between the level of free hydroxyproline and catalase in blood plasma (r=-0.84), and superoxide dismutase (r=-0.79).

DISCUSSION

Periodontal diseases cause not only inflammation of the gums and periodontal ligament but also an imbalance in the regulation of redox processes. Many authors point out that oxidative stress is an important factor in the etiology and pathogenesis of diseases of the oral cavity and teeth [3, 6]. Some studies indicate that neutrophils, lymphocytes, bacteria, smoking, diseases of the cardiovascular system, diabetes mellitus contribute to the formation of reactive oxygen species and provoke the development of oxidative stress in periodontitis. Research by Yang P.S. et al. showed that an increase in superoxide dismutase activity is positively associated with the severity of periodontitis [6]. German researchers have revealed a significant increase in the level of catalase activity when creating a model of hypoxia and inflammation in vitro (p <0.001) [2]. The authors believe that prolonged inflammation causes a decrease in catalase activity, which indicates the formation of an imbalance in the antioxidant defense system, an increase in reactive oxygen species, and the progression of inflammatory diseases of the oral cavity [2]. Also, Oktay S et.al. recorded a high activity of catalase and superoxide dismutase was significantly higher in rats with generalized periodontitis (p <0.001) [7]. Our study showed that catalase and superoxide dismutase activity significantly changes in rats with periodontitis against the background of oxidative stress, which negatively affects the metabolism of collagen in the supporting tissues of the tooth.

CONCLUSION

In rats with periodontitis, the antioxidant defense system is impaired, which is manifested in a decrease in

the activity of the key enzymes of the antioxidant defense activity: catalase and superoxide dismutase. Moreover, the longer the inflammation persists (over three weeks), the lower the catalase activity becomes. Inhibition of the antioxidant system leads to impaired collagen formation and damage to periodontal tissues.

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