

ROLE OF NO IN SOFT PERIODONTAL TISSUES OF RATS DURING STRESS AND INFLAMMATION

PLETNOV V. V., TKACHENKO O. T., MYKYTENKO A. O.

Poltava State Medical University, Ukraine

E-mail: pletnew200211@gmail.com

Received 2023/03/14

Revised 2023/04/24

Accepted 2023/04/28

Bacterial lipopolysaccharide (LPS) induces the formation of nitric oxide (NO) and proinflammatory cytokines by activating nuclear factor κ B (NF- κ B) signaling pathways through toll-like receptor 4 (TLR4), which leads to destruction of periodontal soft tissues, including resorption of alveolar bones [1]. The Scientific School of Professor Tarasenko L.M. substantiated biochemical mechanisms of stress-induced periodontal tissue damage [2]. Chronic stress leads to an imbalance in the immune homeostasis of periodontal tissues, which can lead to the development of chronic periodontitis and/or increase the destruction of biopolymers of periodontal tissues [3].

Glucocorticoids, which are released during chronic stress, can bind to activated NF- κ B — inactivating its action, increase the transcription of the I κ B α gene, which binds to activated NF- κ B, blocking its effect on DNA sites [4].

Aim. To evaluate the activity of NO-synthase isoforms, the concentration of peroxynitrites and nitrosothiols in the soft tissues of the periodontium of rats under the conditions of modeling chronic stress against the background of lipopolysaccharide-induced inflammation.

Methods. Experimental studies were performed on 24 male Wistar rats weighing 190–240 g. The animals were divided into 4 groups: 1 — control, 2 — chronic stress (ChrStr group), animals were kept above water for 1 hour every day for 30 days, 3 — animals that were intraperitoneally injected with 0.4 μ g/kg of bacterial LPS of *S. typhi* (pyrogenal) (LPS group) according to scheme described by Mykytenko A.O. et al. [5]; 4 — animals that were simultaneously simulated chronic stress as in group 2 and administered LPS as in group 3 (ChrStr+LPS). The activity of inducible NO-synthase (iNOS), constitutive NO-synthase (cNOS) (Yelinska A.M., 2019), the concentration of nitrosothiols (S-NO) (Gaston B., 1993), and concentration of peroxynitrites of alkali and alkaline earth metals (ONOO⁻) (Akimov O.Y., 2016) were studied in the homogenate of the periodontal soft tissues of rats. The obtained results were subjected to statistical processing using the Mann-Whitney test.

Results. The activity of iNOS in the soft periodontal tissues of rats under chronic stress simulation conditions was increased 1.44 times compared to control group. The activity of iNOS under the conditions of LPS administration was increased 3.88 times compared to the control group. The activity of iNOS under the conditions of combined exposure to chronic stress and LPS was increased 1.95 times compared to the control group (Fig. 1, A).

The activity of cNOS in the soft periodontal tissues of rats under chronic stress simulation conditions was decreased by 1.04 times compared to the control group. Activity of cNOS in the periodontal soft tissues of rats under the conditions of LPS administration was increased 3.03 times compared to the control group. Activity of cNOS under conditions of combined exposure to chronic stress and LPS was increased 1.53 times compared to the control group (Fig. 1, B).

The concentration of ONOO⁻ in the soft periodontal tissues of rats under chronic stress simulation conditions was increased 1.46 times compared to the control group (Fig. 2, A). The concentration of ONOO⁻ under the conditions of LPS administration was increased by 1.12 times compared to the control group. The concentration of ONOO⁻ under conditions of combined exposure to chronic stress and LPS was increased by 1.39 times compared to the control group.

The concentration of nitrosothiols in the soft periodontal tissues of rats under the conditions of chronic stress simulation was decreased by 6.03 times compared to the control group (Fig. 2, B). The concentration of nitrosothiols in the periodontal soft tissues of rats under the conditions of LPS administration was decreased by 2.73 times compared to the control group. The concentration of nitrosothiols under conditions of combined exposure to chronic stress and LPS was decreased by 3.15 times compared to the control group.

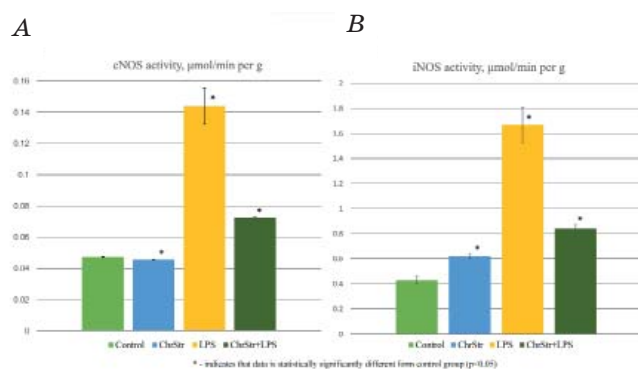


Fig. 1. The activity of cNOS (A) and iNOS (B) in the periodontal soft tissues of rats under the conditions of chronic stress simulation against the background of lipopolysaccharide-induced inflammation:

* — indicates that data is significantly different compared to control group ($P < 0.05$)

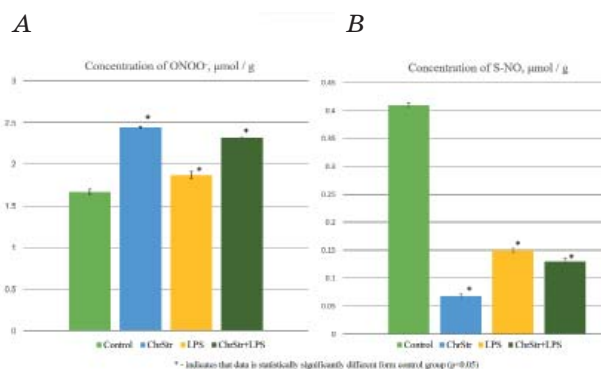


Fig. 2. The concentration of peroxynitrite (A) and nitrosothiols (B) in the periodontal soft tissues of rats under the conditions of chronic stress simulation against the background of lipopolysaccharide-induced inflammation:

* - indicates that data is significantly different compared to control group ($P < 0.05$)

Discussion. The combined effect of chronic stress and bacterial LPS increases iNOS activity relative to the control group due to the interaction of LPS with TLR 4 and the induction of iNOS expression [6]. The combination of chronic stress and bacterial LPS introduction leads to limitation of LPS-induced increase in cNOS and iNOS activities. Simultaneous decrease in cNOS and iNOS activities, observed in this group can lead to lowered concurrence of these enzymes for the substrate of reaction, which in turn decreases chances for cNOS uncoupling. However, the state of cNOS coupling with substrate in our study was not investigated and requires further research. Increase in ONOO⁻ concentration creates a threat of nitrosative stress development, while decrease in S-NO concentration may lead to microcirculatory dysfunction in soft periodontal tissues.

Conclusions. The combined effect of bacterial lipopolysaccharide and chronic stress leads to increased production of nitrogen monoxide from inducible NO-synthase and elevates concentration of reactive forms of nitrogen, which creates possibility for development of nitrosative stress in the soft periodontal tissues.

Keywords: chronic stress; lipopolysaccharide; nitrogen monoxide; periodontal soft tissues.

Authors' contribution. Pletnov V.V. — data gathering, data analysis, data interpretation, writing original draft. Tkachenko O.T. — data gathering, data analysis, data interpretation, writing original draft. Mykytenko A.O. — design and concept, data analysis, statistics, revision and approval of original draft.

REFERENCES

1. Kim S., Lee J. Y., Park J. Y., Kim Y., Kang C. H. Lacticaseibacillus rhamnosus MG4706 Suppresses Periodontitis in Osteoclasts, Inflammation-Inducing Cells, and Ligature-Induced Rats. *Nutrients*. 2022, 14(22), 4869. <https://doi.org/10.3390/nu14224869>
2. Tarasenko L.M., Petrushanko T.A. *Stress and periodontium*. Poltava; 1999. 192 P.
3. Spector A. M., Postolache T. T., Akram F., Scott A. J., Wadhawan A., Reynolds M. A. Psychological Stress: A Predisposing and Exacerbating Factor in Periodontitis. *Curr Oral Health Rep*. 2020, v. 7, p. 208–215. <https://doi.org/10.1007/s40496-020-00282-2>
4. Barnes P. J., Karin M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N. Engl. J. Med.* 1997, 336(15), 1066-1071. <https://doi.org/10.1056/NEJM199704103361506>
5. Mykytenko A. O., Akimov O. Y., Neporada K. S. Influence of lipopolysaccharide on the development of oxidative-nitrosative stress in the liver of rats under conditions of chronic alcohol intoxication. *Fiziol Zh*. 2022, 68(2), 29–35. : <https://doi.org/10.15407/fz68.02.029>
6. Jin S. J., Song Y., Park H. S., Park K. W., Lee S., Kang H. Harmine Inhibits Multiple TLR-Induced Inflammatory Expression through Modulation of NF-κB p65, JNK, and STAT1. *Life (Basel)*. 2022, 12(12), 2022. <https://doi.org/10.3390/life12122022>