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Effect of lipopolysaccharide on the development of oxidative-nitrosative stress in salivary glands and soft periodontal tissues of rats under conditions of water avoidance stress

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ABSTRACT

Introduction and aim. Violation in the salivary glands will inevitably cause changes in periodontium, and periodontitis can disrupt the functioning of the salivary glands. The purpose of the work is to evaluate changes in NO-synthase and arginase activities, pro- and antioxidant balance in rat salivary glands and soft periodontal tissues during administration of bacterial lipopolysaccharide (LPS) and water avoidance stress (WAS) modeling.

Material and methods. The experiment was performed on 24 rats. The animals were divided into 4 groups: control, WAS, animals injected intraperitoneally with 0.4 μg/kg of bacterial LPS of *Salmonella typhi*, WAS+LPS.

Results. Water avoidance stress led to decrease of inducible NO-synthase (iNOS) activity in salivary glands by 1.63 times, but decreased arginase activity by 1.15 times, superoxide production increased by 1.53 times, catalase activity decreased by 1.2 times, and malonic dialdehyde (MDA) increased by 1.19 times compared to the control. Lipopolysaccharide led to increase of constitutive NO-synthases (cNOS) activity in salivary glands by 1.48 times, but decreased arginase activity by 6.15 times, catalase activity increased by 2.6 times and superoxide dismutase activity decreased by 2.74 times, and MDA increased by 6.84 times compared to the control. Water avoidance stress + LPS in salivary glands led to decrease of cNOS and arginase activity by 1.09 and 1.19 times, respectively, superoxide production increased by 1.88 times, catalase and

superoxide dismutase activity decreased by 1.06 times and 1.34 times, respectively, and MDA increased by 2.44 times compared to the control.

Water avoidance stress led to increase of iNOS activity in periodontium by 1.44 times and arginase activity decreased by 1.37 times, superoxide production increased 1.32 times, catalase activity and superoxide dismutase activities decreased by 1.27 times and by 1.53 times, respectively, and MDA increased by 1.31 times compared to the control. Lipopolysaccharide led to increase of iNOS activity in the periodontium by 3.88 times, arginase activity decreased by 2.69 times, superoxide production increased 1.64 times, catalase activity increased by 4.32 times, and MDA increased by 4.51 times compared to the control. Water avoidance stress + LPS in periodontium led to increase of iNOS and cNOS activities by 1.95 times and 1.53, respectively, arginase activity decreased by 1.39 times, superoxide production increased 1.66 times, catalase activity increased by 1.11 times, and MDA increased by 1.53 times compared to the control.

Conclusion. The combination of LPS and WAS leads to changes in NO production and oxidative stress in salivary glands and the periodontium.

Keywords. bacterial lipopolysaccharide, nitric oxide, salivary glands, soft periodontal tissues, water avoidance stress

Introduction

Salivary glands and the periodontium are part of a single functional system that forms homeostasis in the oral cavity. Patients with generalized chronic periodontitis have an increased risk of developing primary Sjogren's syndrome.[1] Violation of saliva secretion caused by Sjogren's syndrome leads to increased inflammatory processes in periodontal tissues.[2,3] The functional state of salivary glands and the periodontium are interconnected through common metabolic patterns. For instance, excessive amount of palmitic acid in blood can lead both to Sjogren's syndrome development and inflammation in the periodontium.[2] Terrizzi et al. in their study showed that during adaptation to hypoxia in salivary glands, secretion drops, while levels of prostaglandin E_2 increase.[3] Increased levels of prostaglandin E_2 in turn lead to excessive alveolar bone loss, thus mimicking the bone loss observed during periodontitis.[3] Lin et al. showed in their research that changes in oral microbiota caused by xerostomia form a vicious circle and exacerbate xerostomia further.[4]

Thus, a violation of the physiological functioning of the salivary glands will inevitably cause changes in the functional state of the periodontal tissues, and periodontitis can disrupt the functioning of the salivary glands.

Lipopolysaccharide (LPS) is an important factor in the virulence of gram-negative bacteria and a powerful activator of innate and adaptive immune responses, as well as being capable of triggering intracellular signaling of tissue destruction. It plays an important role in the pathogenesis of periodontitis, where a large number of gram-negative species is a typical determinant of the periodontal microbiota. Translocation of

LPS into the circulation causes endotoxemia, which is involved in the pathogenesis of many inflammatory conditions, including atherosclerotic cardiovascular disease, obesity, liver disease, diabetes, metabolic syndrome, and oral inflammatory diseases, and is therefore considered a risk factor. It is known that patients with periodontitis have an increased concentration of circulating LPS and metabolic disturbances, which can be the cause or consequence of endotoxemia.[5] Bacterial endotoxin causes inflammation in the salivary glands, which leads to impaired secretion of saliva.[6,7]

Systemic inflammatory response can change composition of saliva drastically, leading to absence of proteins necessary for oral homeostasis.[8] Recent studies have revealed the role of small extracellular vesicles in saliva as powerful diagnostic tools for periodontitis development, hinting of presence of a special interconnection between metabolism of the periodontium and salivary glands.[9] The presence of oxidative stress markers in saliva corresponds to development of oxidative stress in the periodontium, however, whether salivary glands are involved in increase of oxidative stress biomarkers in saliva is still under question.[10]

The Scientific School of Professor Tarasenko substantiated biochemical mechanisms of stress-induced damage to salivary glands and periodontal tissues.[11] Stress causes drastic changes in the composition of saliva, characterized by changes in the electrolyte composition, protein secretion, and an increase in inflammatory markers.[12,13] Psychological stress leads to an imbalance in the immune homeostasis of periodontal tissues, which can lead to the development of chronic periodontitis and/or an increase in the destruction of biopolymers of periodontal tissues.[14]

The combined effect of chronic stress and bacterial LPS on periodontal soft tissues and salivary glands is poorly understood.

Aim

To evaluate changes in the activity of NO-synthase and arginase, pro- and antioxidant balance in salivary glands and soft periodontal tissues of rats under the conditions of administration of bacterial lipopolysaccharide against the background of water avoidance stress modeling.

Material and methods

Ethical approval

Research was conducted in accordance with the standards of the Council of Europe Convention on Bioethics "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (1997), general ethical principles of experiments on animals approved by the First National Congress on Bioethics of Ukraine (September 2001) and other international agreements and national legislation in this area. The rats were kept in a vivarium accredited in accordance with the "Standard rules of order, equipment and maintenance of experimental biological clinics (vivarium)." All

experimental procedures were approved by Bioethical Committee of Poltava State Medical University (Record № 212 from 27.01.2023).

Sample and experimental groups

The experiment was performed on 24 sexually mature male Wistar albino rats weighing 190–240 g. The animals were divided into 4 groups of 6 rats.

I – control group, intact animals, which were intraperitoneally injected with 0.2 mL of a 0.9% aqueous solution of sodium chloride.

II – water avoidance stress group (WAS group), simulated stress according to the water avoidance stress modelling protocol.

III – a group of rats injected with lipopolysaccharide (LPS group) according to pyrogenal injection protocol.

IV – group of rats intraperitoneally injected with pyrogenal (WAS+LPS group) that were subjected to the water avoidance stress modelling protocol and pyrogenal injection protocols.

The conditions for keeping animals in the vivarium were standard. Animals were removed from the experiment on the 30th day and blood sampling was performed from the right ventricle of the heart under thiopental anesthesia.

Water avoidance stress modelling protocol

The rats were placed on a platform (8×6 cm) in the middle of a plastic container with a diameter of 90 cm and height of 50 cm filled with water of 25°C to 1 cm below the level of the platform. Rats avoided water by staying on the platform for 1 hour during 30 days.[15]

Pyrogenal injection protocol

Bacterial LPS of *Salmonella typhi* (pyrogenal) was injected intraperitoneally with 0.4 μg/kg of in the first week 3 times and then once a week throughout the duration of the experiment.[16,17]

Biochemical analysis

For biochemical analysis, we used 10% periodontal soft tissues and salivary gland homogenate and blood serum. Periodontal soft tissues and salivary gland homogenate was obtained after homogenization of 1 g of rat periodontal soft tissues or 1 g of salivary gland with 9 mL of 0.2 M Tris-buffer solution (Trisaminomethane-hydrochloric acid buffer, pH=7.4). Then it was centrifuged at 3000 g for 10 minutes. The upper layer (supernantant) was used for further biochemical analysis. Blood plasma was obtained after addition of 0.109 M sodium citrate at ratio 9:1 and subsequent centrifugation at 3000 g for 10 minutes.

Cortisol concentration was determined in the aliquot of 0.1 mL of blood plasma of rats.

To determine the concentration of cortisol, 2 mL of ammonium tetramethylhydroxide pentahydrate solution (100 mg of ammonium tetramethylhydroxide pentahydrate was dissolved in 5 mL of distilled water, then 5 mL of the resulting solution was mixed with 45 mL of methyl alcohol) and 2 ml of nitroblue tetrazolium chloride solution (100 mg of nitroblue tetrazolium chloride in 50 mL of methyl alcohol). As a result, a red-colored dye was formed with a maximum light absorption at a wavelength of 510 nm.[18]

Total NO-synthase (gNOS) activity was evaluated by the increase of nitrites after incubation of 10% liver homogenate (0.2 mL) for 30 min in the incubation solution (2.5 mL of 0.1 M trisbuffer, 0.3 mL of 320 mM aqueous solution of L-arginine and 0.1 ml of 1 mM $NADPH^+H^+$ solution). To determine the activity of constitutive NOS (cNOS) 1% solution of aminoguanidine hydrochloride was used and the incubation time was extended to 60 min.[19,20] The activity of inducible NOS (iNOS) was calculated by the formula: iNOS=gNOS-cNOS.

Adrenaline auto-oxidation reaction in an alkaline environment with the generation of superoxide was used to determine SOD activity. SOD activity was calculated in conventional units (c.u., 1 unit indicates a 50% inhibition of the reaction rate) by comparison of speed of adrenaline auto-oxidation in presence of tissue homogenate and without it.[21]

The method of catalase activity estimation was based on the determination of colored products formed by the reaction of hydrogen peroxide with ammonium molybdate. The amount of hydrogen peroxide decomposed in the presence of a sample containing catalase can help us to make a conclusion about the activity of catalase.[21]

Free malonic dialdehyde (MDA) specifically reacts with 1-methyl-2-phenyl-indole in a mixture of methanol and acetonitrile to form chromogen (carbocyanine dye) with a maximum light absorption at a wavelength of 586 nm.[22]

Peroxynitrite concentration was measured by using its reaction with potassium iodide under pH 7.0 in 0.2 M phosphate buffer with the same pH, which yields I_3 with maximum absorbance at 355 nm wavelength.[19]

The method for the determination of nitrosothiols was based on the determination of the difference in the concentration of nitrites (NO_2^-) using Griess reagent (modified by Ilosvay) before and after oxidation of nitrosothiol complexes (SNO) to nitrites with a solution of mercuric chloride ($HgCl_2$).[23]

Sulfides specifically react with N-N-dimethyl-para-phenylenediamine in the presence of Fe^{3+} ions and excess of hydrochloric acid to form a red-pink chromogen with a maximum light absorption at a wavelength of 667 nm.[24]

The method for estimation of superoxide anion radical production was based on nitroblue tetrazolium (NBT) reduction by superoxide with the formation of diformazan, a dark blue insoluble precipitate.[24]

OMP were measured by definition of additional carbonyl groups in side chains of amino acids by reaction of 2,4-dinitrophenylhydrazine with carbonyl groups.[25]

Statistical analysis

Statistical processing of the results of biochemical studies was carried out using a pairwise comparison using the non-parametric Mann-Whitney method. All statistical calculations were performed in the Microsoft office Excel program and its extension Real Statistics 2019 developed by Charles Zaiontz. The difference was considered statistically significant at $p<0.05$.

Results

The level of cortisol in the blood of experimental animals is shown in Fig. 1.

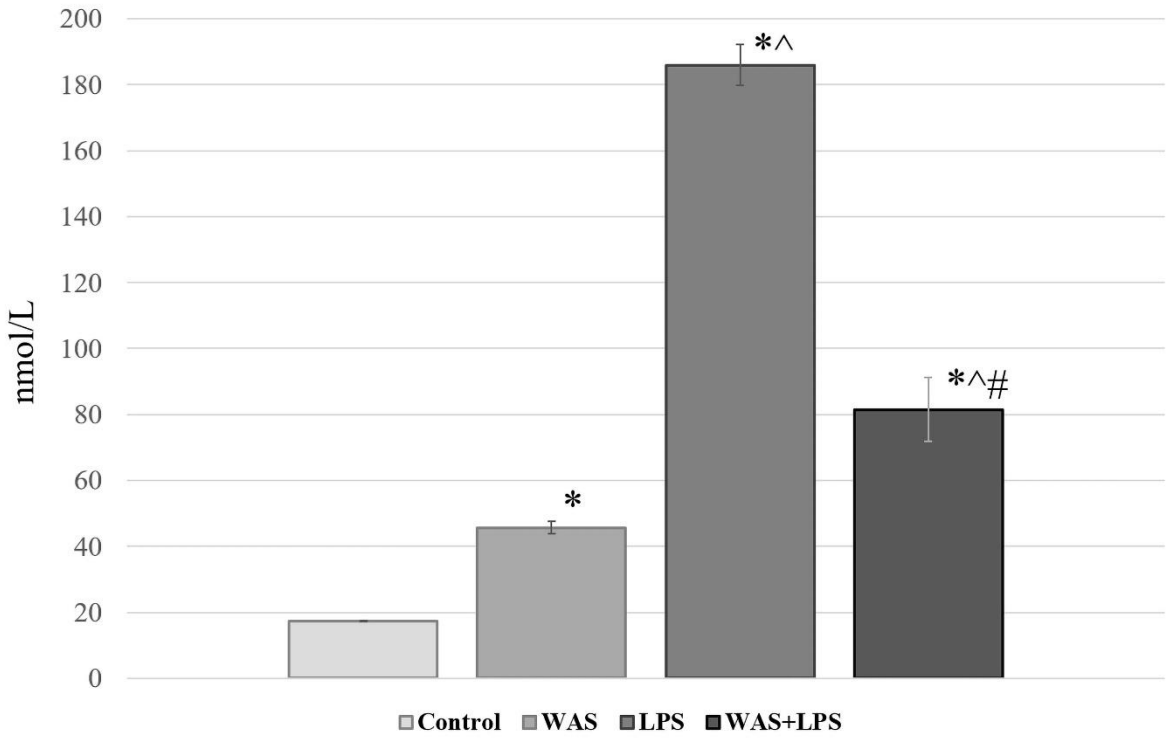

Fig. 1. Cortisol concentration in the blood of rats under the conditions of administration of bacterial lipopolysaccharide against the background of water avoidance stress modeling, M±m, * – indicates that data is statistically significantly different form control group ($p<0.05$), ^ – indicates that data is statistically significantly different form WAS group ($p<0.05$), # – indicates that data is statistically significantly different form LPS group ($p<0.05$), WAS – water avoidance stress, LPS – lipopolysaccharide

Biochemical changes in the salivary glands of rats under the conditions of administration of bacterial lipopolysaccharide against the background of water avoidance stress modeling

The activity of iNOS in the salivary glands of rats under the conditions of modeling water avoidance stress decreased by 1.63 times compared to the control group of animals. The activity of iNOS in the salivary glands of rats under the conditions of LPS administration increased 2 times compared to the group of rats

with stress syndrome. The activity of iNOS in salivary glands of rats under conditions of combined exposure to water avoidance stress and administration of LPS increased by 1.35 times compared to the group of rats with stress syndrome and decreased by 1.48 times compared to the group of rats that were administered LPS (Table 1).

Table 1. Biochemical changes in the salivary glands of rats under the conditions of administration of bacterial lipopolysaccharide against the background of water avoidance stress modeling (M±m)[a]

Parameters	**Control group**	**WAS group**	**LPS group**	**WAS+LPS group**
Inducible NO-synthase, μmol/min per g of protein	0.8±0.08	0.49±0.02*	0.98±0.07^	0.66±0.03^#
Constitutive NO-synthase, μmol/min per g of protein	0.0614±0.0002	0.0591±0.0006*	0.0908±0.0004*^	0.0564±0.0005*^#
Arginase activity, μmol/min per g of protein	1.66±0.014	1.91±0.03*	0.27±0.04*^	1.39±0.003*^#
S-NO, μmol/g	0.29±0.0008	0.095±0.003*	0.84±0.08*^	0.13±0.0042*^#
$ONOO^-$, μmol/g	2.17±0.04	3.8±0.11*	6.08±0.17*^	4.31±0.13*^#
NO_2^- concentration, nmol/g	7.9±0.11	6.28±0.17*	36.38±0.23*^	6.59±0.28*#
Superoxide anion radical production, nmol/s per g	0.64±0.004	0.98±0.004*	0.78±0.08^	1.2±0.004*^#
Catalase activity, μkat/g	0.55±0.004	0.46±0.004*	1.43±0.01*^	0.52±0.007*^#
Superoxide dismutase activity, c.u.	2.11±0.07	0.77±0.03*	2.77±0.35^	1.58±0.19*^#
Malondialdehyde concentration, μmol/g	6.26±0.04	13.73±0.1*	42.83±4.37*^	15.28±0.06*^#
OMP, c.u.	0.034±0.0015	0.091±0.0009*	0.0802±0.002*^	0.105±0.0012*^#
Sulfide anion concentration, μmol/g	8.17±0.103	21.16±0.42*	28.4±0.1*^	17.92±0.08*^#

[a] * – indicates that data is statistically significantly different form control group ($p<0.05$), ^ – indicates that data is statistically significantly different form WAS group ($p<0.05$), # – indicates that data is statistically significantly different form LPS group ($p<0.05$), WAS – water avoidance stress, LPS – lipopolysaccharide

The activity of cNOS in the salivary glands of rats under the conditions of modeling water avoidance stress decreased by 0.1 times compared to the control group of animals. cNOS activity in the salivary glands of rats under the conditions of LPS administration increased 1.48 times compared to the control group of animals and 1.54 times compared to the group of rats with stress syndrome. cNOS activity under conditions of combined exposure to WAS and LPS administration decreased by 1.09 times compared to the control group of animals, by 1.05 times compared to the group of rats with stress syndrome, and by 1.61 times compared to the group of rats that were administered LPS.

The activity of arginase in the salivary glands of rats under the conditions of modeling water avoidance stress increased 1.15 times compared to the control group of animals. The activity of arginase in the salivary glands of rats under the conditions of LPS administration decreased by 6.15 times compared to the control group of animals and by 7.07 times compared to the group of rats with stress syndrome. The activity of arginase in the salivary glands of rats under conditions of combined exposure to water avoidance stress and LPS administration decreased by 1.19 times compared to the control group and by 1.37 times compared to the group with stress syndrome and increased by 5.15 times compared to the group of rats, who were administered LPS.

The concentration of nitrosothiols in the salivary glands of rats under the conditions of modeling water avoidance stress decreased by 3.05 times compared to the control group of animals. The concentration of nitrosothiols in the salivary glands of rats under the conditions of LPS administration increased 2.9 times compared to the control group of animals and 8.84 times compared to the group of rats with stress syndrome. The concentration of nitrosothiols in the salivary glands of rats under conditions of combined exposure to water avoidance stress and administration of LPS decreased by 2.23 times compared to the control group and by 6.46 times compared to the group of animals administered LPS and increased by 1.37 times compared to the group with stress syndrome.

The concentration of $ONOO^-$ in the salivary glands of rats under the conditions of modeling water avoidance stress increased 1.75 times compared to the control group of animals. The concentration of $ONOO^-$ in the salivary glands of rats under the conditions of LPS administration increased 2.8 times compared to the control group of animals and 1.6 times compared to the group of rats with stress syndrome. The concentration of $ONOO^-$ in the salivary glands of rats under conditions of combined exposure to water avoidance stress and administration of LPS increased by 1.99 times compared to the control group and by 1.13 times compared to the group with stress syndrome and decreased by 1.41 times compared to the group animals that were injected with LPS.

The concentration of NO_2 in the salivary glands of rats under the conditions of modeling water avoidance stress decreased by 1.26 times compared to the control group of animals. The concentration of NO_2 in the salivary glands of rats under the conditions of LPS administration increased 4.61 times compared to the control group of animals and 5.79 times compared to the group of rats with stress syndrome. The concentration of NO_2 in the salivary glands of rats under conditions of combined exposure to water avoidance stress and administration of LPS decreased by 1.2 times compared to the control group and by 5.52 times compared to the group of animals that were administered LPS.

Analyzing antiradical protection and production of reactive oxygen species (ROS) under the conditions of combined exposure to water avoidance stress and LPS administration, we found that SAR production in the salivary glands of rats under the conditions of water avoidance stress simulation increased 1.53 times compared to the control group of animals. SAR production in the salivary glands of rats under the conditions of LPS administration decreased by 1.26 times compared to the group of rats with stress syndrome. SAR production in the salivary glands of rats under conditions of combined exposure to water avoidance stress and LPS administration increased 1.88 times compared to the control group of rats, 1.22 times compared to the group with stress syndrome, and 1.54 times compared to the group of rats , who were administered LPS.

Catalase activity in the salivary glands of rats under the conditions of modeling water avoidance stress decreased by 1.2 times compared to the control group of animals. Catalase activity in the salivary glands of rats under the conditions of LPS administration increased 2.6 times compared to the control group of animals and 3.11 times compared to the group of rats with stress syndrome. Catalase activity in the salivary glands of rats under conditions of combined exposure to water avoidance stress and administration of LPS decreased by 1.06 times compared to the control group of rats and by 2.75 times compared to the group of animals administered LPS and increased by 1.13 times compared to group with stress syndrome.

The activity of SOD in the salivary glands of rats under the conditions of LPS simulation decreased by 2.74 times compared to the control group of animals. The activity of SOD in the salivary glands of rats under the conditions of LPS administration increased 3.6 times compared to the group of rats with stress syndrome. The activity of SOD in the salivary glands of rats under conditions of combined exposure to water avoidance stress and LPS administration decreased by 1.34 times compared to the control group of rats and by 1.75 times compared to the group of animals administered LPS and increased by 2.05 times compared to group with stress syndrome.

The concentration of MDA in the salivary glands of rats under the conditions of modeling water avoidance stress increased by 1.19 times compared to the control group of animals. The concentration of MDA in the salivary glands of rats under the conditions of LPS administration increased 6.84 times compared to the control group of animals and 3.12 times compared to the group of rats with stress syndrome. The concentration of MDA in the salivary glands of rats under conditions of combined exposure to water

avoidance stress and LPS administration increased 2.44 times compared to the control group and 1.11 times compared to the group with stress syndrome and decreased 2.8 times compared to the group of animals, who were administered LPS.

The content of OMP in the salivary glands of rats under the conditions of modeling water avoidance stress increased 2.68 times compared to the control group of animals. The content of OMP in the salivary glands of rats under the conditions of LPS administration increased by 2.36 times compared to the control group of animals and decreased by 1.13 times compared to the group of rats with stress syndrome. The content of OMP in the salivary glands of rats under conditions of combined exposure to water avoidance stress and LPS administration increased 3.09 times compared to the control group of animals, 1.15 times compared to the group with stress syndrome and 1.31 times compared to the group of animals, who were administered LPS.

The concentration of sulfide anion in the salivary glands of rats under the conditions of modeling water avoidance stress increased by 2.59 times compared to the control group of animals. The concentration of sulfide anion in the salivary glands of rats under the conditions of LPS administration increased by 3.48 times compared to the control group of animals and by 1.34 times compared to the group of rats with stress syndrome. The concentration of sulfide anion in the salivary glands of rats under conditions of combined exposure to water avoidance stress and LPS administration increased 2.19 times compared to the control group and decreased 1.18 times compared to the group with stress syndrome and 1.58 times compared to a group of animals that were injected with LPS.

Biochemical changes in the soft periodontal tissues of rats under the conditions of administration of bacterial lipopolysaccharide against the background of water avoidance stress modeling

The activity of iNOS in the soft periodontal tissues of rats under the conditions of modeling water avoidance stress increased by 1.44 times compared to the control group of animals. The activity of iNOS in the periodontal soft tissues of rats under the conditions of LPS administration increased by 3.88 times compared to the control group of animals and by 2.69 times compared to the group of rats with stress syndrome. The activity of iNOS in the periodontal soft tissues of rats under conditions of combined exposure to water avoidance stress and LPS administration increased by 1.95 times compared to the control group of animals and by 1.35 times compared to the group of rats with stress syndrome and decreased by 1.99 times compared to the group of rats that were injected with LPS (Table 2).

Table 2. Biochemical changes in the soft periodontal tissues of rats under the conditions of administration of bacterial lipopolysaccharide against the background of water avoidance stress modeling (M±m)[a]

Parameters	**Control group**	**WAS group**	**LPS group**	**WAS+LPS group**
Inducible NO-synthase, μmol/min per g of protein	0.43±0.03	0.62±0.02*	1.67±0.14*^	0.84±0.03*^#
Constitutive NO-synthase, μmol/min per g of protein	0.0474±0.0002	0.0456±0.0002*	0.1438±0.0115*^	0.0724±0.0007*^#
Arginase activity, μmol/min per g of protein	1.75±0.003	1.28±0.02*	0.65±0.01*^	1.26±0.01*#
S-NO, μmol/g	0.41±0.004	0.068±0.005*	0.15±0.003*^	0.13±0.0005*^#
$ONOO^-$, μmol/g	1.67±0.03	2.44±0.01*	1.87±0.04*^	2.32±0.007*^#
NO_2^- concentration, nmol/g	3.14±0.06	4.26±0.11*	22.04±0.23*^	3.65±0.11*^#
Superoxide anion radical production, nmol/s per g	0.82±0.03	1.08±0.008*	0.5±0.01*^	1.36±0.01*^#
Catalase activity, μkat/g	0.38±0.001	0.3±0.001*	1.64±0.008*^	0.42±0.001*^#
Superoxide dismutase activity, c.u.	6.36±0.47	4.17±0.18*	9.86±1.29^	6±0.32^
Malondialdehyde concentration, μmol/g	9.4±0.1	12.32±0.13*	42.39±0.22*^	14.41±0.1*^#
OMP, c.u.	0.027±0.0004	0.077±0.0004*	0.048±0.0004*^	0.097±0.0006*^#
Sulfide anion concentration, μmol/g	1.81±0.06	4.19±0.05*	4.11±0.04*	2.57±0.04*^#

[a] * – indicates that data is statistically significantly different form control group ($p<0.05$), ^ – indicates that data is statistically significantly different form WAS group ($p<0.05$), # – indicates that data is statistically significantly different form LPS group ($p<0.05$), WAS – water avoidance stress, LPS – lipopolysaccharide

The activity of cNOS in the soft periodontal tissues of rats under the conditions of modeling water avoidance stress decreased by 1.04 times compared to the control group of animals. cNOS activity in the periodontal soft tissues of rats under the conditions of LPS administration increased by 3.03 times compared to the control group of animals and by 3.15 times compared to the group of rats with stress syndrome. cNOS activity in the periodontal soft tissues of rats under conditions of combined exposure to water avoidance stress and LPS administration increased by 1.53 times compared to the control group of animals, by 1.59 times compared to the group of rats with stress syndrome, and decreased by 1.99 times compared to the group of rats that were injected with LPS.

The activity of arginase in the soft periodontal tissues of rats under the conditions of modeling water avoidance stress decreased by 1.37 times compared to the control group of animals. The activity of arginase in the soft periodontal tissues of rats under the conditions of LPS administration decreased by 2.69 times compared to the control group of animals and by 1.97 times compared to the group of rats with stress syndrome. The activity of arginase in the soft periodontal tissues of rats under conditions of combined exposure to water avoidance stress and administration of LPS decreased by 1.39 times compared to the control group and increased by 1.94 times compared to the group of rats that were administered LPS.

The concentration of nitrosothiols in the soft periodontal tissues of rats under the conditions of modeling water avoidance stress decreased by 6.03 times compared to the control group of animals. The concentration of nitrosothiols in the periodontal soft tissues of rats under the conditions of LPS administration decreased by 2.73 times compared to the control group of animals and increased by 2.21 times compared to the group of rats with stress syndrome. The concentration of nitrosothiols in the soft periodontal tissues of rats under conditions of combined exposure to water avoidance stress and administration of LPS decreased by 3.15 times compared to the control group and by 1.15 times compared to the group of animals injected with LPS and increased by 1.91 times compared to the group with stress syndrome.

The concentration of $ONOO^-$ in the soft periodontal tissues of rats under the conditions of modeling water avoidance stress increased by 1.46 times compared to the control group of animals. The concentration of $ONOO^-$ in the periodontal soft tissues of rats under the conditions of LPS administration increased by 1.12 times compared to the control group of animals and decreased by 1.3 times compared to the group of rats with stress syndrome. The concentration of $ONOO^-$ in the soft periodontal tissues of rats under conditions of combined exposure to water avoidance stress and administration of LPS increased by 1.39 times compared to the control group and by 1.24 times compared to the group of animals injected with LPS and decreased by 1.05 times compared to the group with stress syndrome.

The concentration of NO_2 in the soft periodontal tissues of rats under the conditions of modeling water avoidance stress decreased by 1.36 times compared to the control group of animals. The concentration of NO_2 in the periodontal soft tissues of rats under the conditions of LPS administration increased by 7.02 times compared to the control group of animals and by 5.17 times compared to the group of rats with stress

syndrome. The concentration of NO_2 in the soft periodontal tissues of rats under conditions of combined exposure to water avoidance stress and LPS administration increased by 1.16 times compared to the control group and decreased by 1.17 times compared to the group with stress syndrome and by 6.04 times compared to with a group of animals that were injected with LPS.

SAR production in the soft periodontal tissues of rats under water avoidance stress simulation conditions increased by 1.32 times compared to the control group of animals. SAR production in the periodontal soft tissues of rats under the conditions of LPS administration decreased by 1.64 times compared to the control group of animals and by 2.16 times compared to the group of rats with stress syndrome. SAR production in the periodontal soft tissues of rats under conditions of combined exposure to water avoidance stress and LPS administration increased by 1.66 times compared to the control group of rats, by 1.26 times compared to the group with stress syndrome and by 2.72 times compared to with a group of rats that were injected with LPS.

Catalase activity in the soft periodontal tissues of rats under water avoidance stress simulation conditions decreased by 1.27 times compared to the control group of animals. Catalase activity in the periodontal soft tissues of rats under the conditions of LPS administration increased by 4.32 times compared to the control group of animals and by 5.47 times compared to the group of rats with stress syndrome. Catalase activity in the soft periodontal tissues of rats under conditions of combined exposure to water avoidance stress and LPS administration increased by 1.11 times compared to the control group of rats and by 1.4 times compared to the group with stress syndrome and decreased by 3.9 times compared to the group of animals that were injected with LPS.

The activity of SOD in the soft periodontal tissues of rats under the conditions of modeling water avoidance stress decreased by 1.53 times compared to the control group of animals. The activity of SOD in the soft periodontal tissues of rats under the conditions of LPS administration increased by 2.36 times compared to the group of rats with stress syndrome. The activity of SOD in the soft periodontal tissues of rats under conditions of combined exposure to water avoidance stress and administration of LPS decreased by 1.64 times compared to the group of animals with stress syndrome.

The concentration of MDA in the soft periodontal tissues of rats under the conditions of modeling water avoidance stress increased by 1.31 times compared to the control group of animals. The concentration of MDA in the periodontal soft tissues of rats under the conditions of LPS administration increased by 4.51 times compared to the control group of animals and by 3.44 times compared to the group of rats with stress syndrome. The concentration of MDA in the periodontal soft tissues of rats under conditions of combined exposure to water avoidance stress and LPS administration increased by 1.53 times compared to the control group and by 1.17 times compared to the group with stress syndrome and decreased by 2.94 times compared to with a group of animals that were injected with LPS.

The content of OMP in the soft periodontal tissues of rats under the conditions of modeling water avoidance stress increased by 2.85 times compared to the control group of animals. The content of OMP in the periodontal soft tissues of rats under the conditions of LPS administration increased by 1.78 times compared to the control group of animals and decreased by 1.6 times compared to the group of rats with stress syndrome. The content of OMP in the periodontal soft tissues of rats under conditions of combined exposure to water avoidance stress and LPS administration increased by 3.59 times compared to the control group of animals, by 1.26 times compared to the group with stress syndrome and by 2.02 times compared to with a group of animals that were injected with LPS.

The concentration of sulfide anion in the soft periodontal tissues of rats under the conditions of modeling water avoidance stress increased by 2.31 times compared to the control group of animals. The concentration of sulfide anion in the soft periodontal tissues of rats under the conditions of LPS administration increased by 2.27 times compared to the control group of animals. The concentration of sulfide anion in the periodontal soft tissues of rats under conditions of combined exposure to water avoidance stress and LPS administration increased by 1.42 times compared to the control group of rats and decreased by 1.63 times compared to the group with stress syndrome and by 1. 6 times compared to the group of animals that were injected with LPS.

Discussion

Role of nitric oxide and competitive L-arginine metabolism in physiology and pathology of oral cavity

Nitric oxide has an antibacterial properties regarding periodontal pathogens.[26] These properties are connected to ability of nitric oxide to transform to nitrite (NO_2^-) and peroxynitrite ($ONOO^-$) depending on the state of oxygen involved in the reaction. Simple oxygen (O_2) reacts with NO resulting in NO_2^- formation, while superoxide anion radical, as an active form of oxygen ($O_2^{\cdot-}$), reacts with NO with $ONOO^-$ formation. Both these reactive forms of nitrogen can cause nitration of bacterial proteins.[27] In periodontium the main producer of NO is iNOS derived from tissue macrophages polarized by pro-inflammatory (M1) phenotype.[28] Arginases metabolize L-arginine to L-ornithine, which is in turn transformed by ornithine decarboxylase to putrescine and other polyamines. Polyamines are potent stimulants of cell division and are necessary for high speed regeneration of tissues of oral cavity. However, increased activity of arginase during inflammation may have adverse effects, because it signifies the shift in macrophage polarization by anti-inflammatory (M2) phenotype, which will impede host defense mechanisms related to nitric oxide production.[29] Therefore iNOS/Arginase ration can be used as a marker of predominance of macrophage polarization in specific tissues, because both enzymes are markers of different states of macrophages.[30] Increased expression of iNOS on the background of decreased expression of arginase evidences about predominant M1 polarization of tissue macrophages, an active stage of host immune response and increased exudation.[31] Vice versa, increased expression of arginase on the background of decreased expression of

iNOS evidences about predominant M2 polarization of tissue macrophages, a regeneratory stage of inflammation and decreased immune response.[32]

Mechanisms of influence of water avoidance stress on biochemical parameters in salivary glands and soft periodontal tissues

Simulation of water avoidance stress leads to a decrease in nitric oxide production by iNOS in the salivary glands, which may be related to the inhibitory effect of the increased cortisol concentration observed in this group on the activation of the transcription factor NF-κB.[33] It is worth noting that our results differ from those of other scientists regarding the effect of water avoidance stress on iNOS activity.[34,35] The decrease in iNOS activity observed in our studies may be related to a longer (30 days) duration of water avoidance stress simulation, which will lead to the development of the "exhaustion" stage of the general adaptation syndrome, in contrast to the 10-day simulation, where mechanisms may prevail, characteristic of the "maximum adaptation" stage of the general adaptation syndrome. It is also worth noting that in the soft tissues of the periodontium, we observed an increase in the activity of iNOS. This may indicate a higher rate of exhaustion of adaptive capabilities in the salivary glands, when compared with the soft tissues of the periodontium. Another mechanism underlying the described changes in iNOS activity in salivary glands under conditions of water avoidance stress may be the characteristics of resident salivary gland macrophages, which have a greater tendency to polarize according to the anti-inflammatory (M2) phenotype, in contrast to resident soft tissue macrophages periodontal tissues.[36]

Changes in the activity of arginases and constitutive NO-synthase isoforms observed in salivary glands and periodontal soft tissues are probably associated with increased competition for the reaction substrate in the iNOS-arginase-cNOS triangle. Among these enzymes, iNOS has the greatest affinity for the substrate, therefore, an increase in iNOS activity leads to a decrease in the activity of arginases in the soft tissues of the periodontium, while in the salivary glands we observe the opposite changes. Constitutive isoforms of NO-synthase have the lowest affinity for the substrate, therefore, with an increase in the activity of either arginase or iNOS, they remain in conditions of substrate deficiency, which causes a decrease in their activity both in the salivary glands and in the soft tissues of the periodontium. This can lead to vasoconstriction of the vessels of the microcirculatory channel of the soft tissues of the periodontium and salivary glands and play a leading role in the development of endothelial dysfunction under these conditions.

In the salivary glands, against the background of a decrease in the concentration of nitric oxide deposited in nitrosothiols and the concentration of nitrites, an increase in the concentration of peroxynitrites is observed during water avoidance stress modeling. Therefore, the nitric oxide required for the formation of peroxynitrite probably does not originate from the L-arginine-dependent pathway of its formation. In the literature, there is no data on the powerful ability of the salivary glands to reduce nitric oxide from nitrates and nitrites that come with food[37]. In the soft tissues of the periodontium, on the contrary, there is a violation

of the utilization of nitric oxide, which is produced in excess as a result of increased iNOS activity, which is evidenced by a decrease in the concentration of nitrosothiols and an increase in the concentration of nitrites and peroxynitrites.

Water avoidance stress is accompanied by increased superoxide anion radical production, depletion of antioxidant systems, and increased oxidative damage to lipids and proteins in salivary glands and soft periodontal tissues. The increased production of SAR may be a consequence of the dissociation of the endothelial isoform of NO-synthase from its substrate, which is confirmed by the reduced activity of cNOS in this group both in the soft periodontal tissues and in the salivary glands.[38] Research shows that the stress hormone corticosterone directly modulates the function of mitochondria, leads to the development of oxidative stress and an increase in the level of homocysteine (toxic to mitochondria). Glucocorticoid receptor signaling also affects mitochondrial function during chronic stress. High levels of ROS (superoxide, hydrogen peroxide and hydroxyl radical) disrupt the functioning of mitochondria[39]. An increase in the concentration of sulfides both in the soft periodontal tissues and in the salivary glands under the conditions of simulation of water avoidance stress may be a reaction of the resident microflora of these tissues aimed at overcoming the consequences of oxidative stress.[40]

Mechanisms of influence of bacterial lipopolysaccharide on biochemical parameters in salivary glands and soft periodontal tissues

The introduction of bacterial LPS leads to an increase in the activity of iNOS against the background of a sharp decrease in the activity of arginases, which releases the substrate for cNOS and explains their increased activity in the salivary glands and soft tissues of the periodontium. This effect of bacterial LPS on L-arginine-dependent enzymes is explained by its ability to promote the polarization of macrophages according to the pro-inflammatory (M1) phenotype and activate the transcription factor NF-κB.[21,41,42] At the same time, an increase in both nitrosothiols, nitrites, and peroxynitrites is observed in the salivary glands under the conditions of increased production of nitric oxide from iNOS upon the introduction of bacterial LPS. In the soft tissues of the periodontium, only the concentration of peroxynitrites and nitrites is increased. An increased concentration of nitrites in the salivary glands and soft tissues of the periodontium may indicate a decrease in the activity of the nitrate-nitrite reductase pathway for the formation of nitric oxide. Although bacterial LPS can induce xanthine oxidase activity and increase nitrite reduction via the dehydrogenase domain of the xanthine oxidoreductase complex, the increased cortisol concentration in this group may negate this effect, since corticosteroids have an inhibitory effect on xanthine oxidase activity.[43,44]

The absence of an increase in the production of SAR in the salivary glands and a decrease in its production in the soft periodontal tissues of rats in the group of animals that were injected with LPS is associated with the accumulation of cortisol in this group of animals, the concentration of which even exceeds that in the WAS group. The accumulation of cortisol is associated with the inhibitory effect of LPS on the activity of

11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), an enzyme that converts cortisol into an inactive form.[45] For activity, 11β-HSD1 requires a cofactor in the form of NAD^+ as an electron acceptor. A decrease in the activity of this enzyme under the influence of LPS will lead to a decrease in the $NADH^+H^+/NAD^+$ ratio, which will reduce the production of SAR from the mitochondrial electron transport chain.[46]

Cortisol is also able to increase the activity of antioxidant enzymes, which explains the changes in the activity of SOD and catalase in salivary glands and soft periodontal tissues under the influence of LPS.[47] It is worth noting that this effect of LPS on SAR production, SOD and catalase activity may depend on the type of bacterial LPS, the organ, and duration of exposure.

The increase in the intensity of LPO and oxidative protein damage observed in the salivary glands and soft tissues of the periodontium in the group of animals injected with LPS indicates the possible initiation of LPO processes by other ROS (hydroxyl radical, hydrogen peroxide, carbonyl radical, etc.) and/or active forms of nitrogen (nitrite radical, peroxynitrite), against the background of insufficient compensation from the enzymatic link of the antioxidant system (SOD and catalase) and an increase in the concentration of sulfide anion (free radical acceptor).

Features of the combined effect of bacterial lipopolysaccharide and water avoidance stress on biochemical indicators in salivary glands and soft periodontal tissues

The combined effect of bacterial LPS and WAS on salivary glands and periodontal soft tissues has an antagonistic effect on iNOS activity, and increases iNOS activity relative to the control group due to LPS exposure. The combined effect of bacterial LPS and WAS also has an antagonistic effect on cNOS and arginase activity and leads to a decrease in cNOS activity and an increase in arginase activity, which corresponds to a WAS-dependent trend. WAS under the conditions of LPS administration to rats provides a decrease in the concentration of nitrosothiols and nitrites and prevents LPS-induced increase in their concentrations.

The combined effect of bacterial LPS and WAS on salivary glands and soft periodontal tissues increases the production of SAR compared to all studied groups, which indicates the predominance of the stimulating effect of WAS on its production. The effect on antioxidant enzymes depends to a greater extent on WAS than from the action of LPS. WAS limits LPO processes when combined with LPS action, but increases damage to protein structures in salivary glands and soft periodontal tissues.

The combined effect of LPS and WAS on the salivary glands and periodontal soft tissues leads to a decrease in the concentration of sulfide anion in comparison with the isolated WAS and LPS exposure groups, which may indicate an increased use of sulfide anion in this group to compensate for the increased production of ROS.

Our study revealed simultaneous changes in salivary glands and soft periodontal tissues during induction of general adaptation syndrome by WAS modelling. Several studies revealed that during general adaptation

syndrome there are functional changes in salivary glands and increased chewing, which is mediated by brain-derived neurotrophic factor (BDNF).[48,49] Elevated chewing may overload tissues of periodontium. At the same time psychological stress can induce changes in bacterial composition of oral microbiota.[50] BDNF during periodontitis is hypermethylated and downregulated, thus its increased concentration during general adaptation syndrome may be a pathogenetic link connecting development of oxidative stress in salivary glands and soft periodontal tissues observed in our study.[51]

Low grade systemic inflammation caused by intraperitoneal administration of bacterial LPS can also cause compensatory increase of BDNF in organs and systems most affected by inflammatory injury, like was shown by Shi J. et al. in their study on example of acute lung injury.[52] However, some scientists consider elevation of BDNF concentration in response to systemic inflammatory states to be an adaptive response aimed at removal of adverse effects caused by inflammation.[53] On the other hand, there are evidences that BDNF can exacerbate inflammatory processes and aggravate damage to organ and tissue.[54] There is information in scientific literature, that prolonged stress can also lead to depletion of BDNF secretion, which subsequently leads to depression and metabolic disorders such as diabetes mellitus type 2, etc.[55]

We did not evaluate the changes in BDNF concentrations in salivary glands and soft periodontal tissues in our study, which makes it a lucrative field for further investigations of mechanisms underlying the changes in production of nitric oxide, activity of antioxidant enzymes and lipid peroxidation observed in our study. Perspectives of further research are not limited to a single factor like BDNF concentration. The role of redox-sensitive transcriptional factors like NF-κB, STAT3, AP-1, Nrf2, p38-MAPK must also be considered and extensively studied, since, as mentioned above, they all or some of them may be either effectors or inductors of changes observed in our study.

Study limitations

The limitations of our study are: small number of animals in study groups, absence of functional tests to evaluate function of salivary glands, absence of assessment of clinical state of periodontium. Another limitation of our study is that we did not assessed the state of oral microbiota, which could have played a major role in observed changes.

Conclusion

Water avoidance stress leads to a decrease in the production of nitric oxide in the salivary glands and to an increase in the production of nitric oxide in the soft periodontal tissues of rats in an L-arginine-dependent manner, increases the formation of reactive forms of oxygen and nitrogen, increases the processes of lipid peroxidation and damage to protein structures.

Bacterial lipopolysaccharide leads to an increase in the production of nitric oxide in an L-arginine-dependent way both in the salivary glands and in the soft tissues of the periodontium, promotes the

formation of both deposited and reactive metabolites of nitric oxide, intensifies the processes of lipid peroxidation and damage to protein structures on against the background of a compensatory increase in the activity of antioxidant enzymes.

The combined effect of bacterial lipopolysaccharide and water avoidance stress leads to a decrease in the production of nitric oxide in the salivary glands and to an increase in its production in the soft tissues of the periodontium of rats in an L-arginine-dependent way, increases the formation of reactive forms of oxygen and nitrogen, enhances the processes of lipid peroxidation and damage to protein structures against the background of exhaustion of the activity of antioxidant enzymes.

Declarations

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Author contributions

Conceptualization, O.A. and A.M.; Methodology, A.M.; Software, O.A.; Validation, V.P., O.T., O.A. and A.M.; Formal Analysis, V.P., O.T., O.A. and A.M.; Investigation, V.P., O.T., O.A. and A.M.; Resources, A.M.; Data Curation, A.M. and O.A.; Writing – Original Draft Preparation, V.P. and O.T.; Writing – Review & Editing, O.A. and A.M.; Visualization, V.P.; Supervision, A.M.; Project Administration, O.A. and A.M.; Funding Acquisition, O.A. and A.M.

Conflicts of interest

Authors declare that there is no known conflict of interest regarding this paper.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

All experimental procedures were approved by Bioethical Committee of Poltava State Medical University (Record № 212 from 27.01.2023).

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