INFLUENCE OF SILICA GEL, CONTAINING NANOPARTICLES OF GOLD AND SILVER, ON THE BIOCHEMICAL INDICES OF THE EXPERIMENTAL INFLAMMATION OF ORAL MUCOSA

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Abstract

Aim: to study the influence of drugs containing nanoparticles of gold and silver on the biochemical markers of inflammation and dysbiosis, under the experimental conditions on the inflammation model of the oral mucosa of rats. **Materials and methods:** the study was conducted on 42 white rats, in which the inflammation of the oral mucosa was experimentally induced by the use of Lipopolysaccharide (LPS). The preliminary application, on the oral mucosa, of the gel containing gold and silver nanoparticles was done 2 days before the experimentally-induced inflammation. The activity of the biochemical indices of inflammation was determined in the homogenate of gingiva and cheek mucosa. **Results and disscusion:** Application of Lipopolysaccharide LPS on the rat oral mucosa caused the growth of elastase activity and MDA content, as a sign of inflammation. Application of gels containing Lyzomucoid with nanoparticles of gold and silver reduced the level of biochemical markers of inflammation, but only in the buccal mucosa. Catalase activity, decreased under the action of LPS, significantly increased in both gingiva and buccal mucosa. **Conclusions**: The gel containing Lyzomucoid preparations and nanoparticles of gold and silver significantly increases the activity of catalase and the antioxidant-prooxidant index (IPA) in both tissues. Decreased levels of oral mucosa dysbiosis were recorded.

Keywords: *oral cavity, lipopolysaccharide, nanoparticles, inflammation, antioxidants, dysbiosis, gels, lyzomucoid.*

1. INTRODUCTION

Relevance of the study. Diseases of the oral mucosa are quite frequent (5-15 %) among other lesions of the oral cavity [1, 2]. Generally, they have an inflammatory nature and diverse clinical manifestations, from exudative inflammation to marked necrotic changes of the oral mucosa [3-5]. Lesions with significant loss of soft tissue of the oral mucosa are difficult to treat, often relapse of the disease appearing [6-8].

A large variety of drugs: antiseptics, enzymes, antibiotics, keratoplastic, etc. are used for the treatment of inflammatory lesions of the oral mucosa, yet data on their efficiency is rather contradictory. Antibacterial agents occupy an important place. The peculiarity of oral mucosa lesions treatment is that opportunistic microflora quickly gets used to antibiotics, which leads to significant changes in microbiota, expressed in the modification of the normal-to-pathogenic organisms ratio.

This situation calls for further improvement of antibacterial drugs, namely the creation of more efficient means, to which microflora will get less accustomed. From this perspective, the use of drugs with gold and silver in the form of nanoparticles looks very promising. Nanoparticles have unique chemical and biological properties essential for therapeutic and preventive actions. Previous studies pf ours have shown the significant antibacterial effect of drugs on the basis of silver nanoparticles [9, 10], as well as their ability to enhance the antibiotic activity of Lyzomucoid, which contains lysozyme and the protease inhibitor ovomukoid [11, 12].

These drugs are adsorbed on fine silica, which greatly enhances their therapeutic effect [13]. Given this, one would assume the beneficial effects of these drugs and of the regeneration processes, including their anti-inflammatory effects and the effects on metabolic processes in the tissues of oral mucosa.

The aim of this study was to investigate the effect of drugs containing nanoparticles of gold and silver on the biochemical indices of inflammation and dysbiosis, under the experimental conditions on the inflammation model of the oral mucosa of rats.

2. MATERIALS AND METHODS

The study was conducted on 42 white rats weighing 180 ± 10 g. Inflammation of the oral mucosa was caused by application of 0.5 ml of gel containing intestinal endotoxin - lipopolysaccharide (LPS) [14, 15]. „Pirogenal" production „Medgamal", Russia, lipopolysaccharide (LPS), derived from *Salmonella typhi*, was used as a drug. The gel was applied on the mucosa, at the rate of 75 mg/kg of body weight of animals for a period of 24 hours.

2 days before, a gel containing 0.5 g nanoparticles of gold (Au) or silver (Ag) was applied on the oral mucosa of the experimental rat. Silica gels containing nanoparticles of gold and silver were provided by the Department of Inorganic Chemistry, "Taras Shevchenko" National University of Kyiv. Gels for mucosa application were prepared by mixing l g of silica gel with nanoparticles of silver and gold, the drug Lyzomucoid (LM), and 95g of 3% sodium carboxymethylcellulose.

The experimental animals were divided into 7 groups - 6 animals in each. In five groups of animals, application of gel containing nanoparticles of silver and gold was carried out in an amount of 0.5 ml per rat for 3 days, 2 days prior to applying the lipopolysaccharide gel (LPS) on the oral mucosa. Group 2 of animals, which received only the gel with LPS, served as the control 1. Group 3 of animals, which received gel Lyzomucoid and LPS, served as the control 2.

The animals were taken out of the experiment on the 4th day (3 days of mucosa gels and 1 day of lipopolysaccharide (LPS) application, under thiopental anesthesia (20 mg / kg) by total bleeding. The mucosa of the gingiva and cheeks was cut and, in its homogenate, the biochemical activity of the inflammation indices - elastase activity [16], content of malondialdehyde [16], activity of antioxidant enzyme catalase – was determined [16]. The ratio of catalase activity to the content of malondialdehyde (MDA) prooxidant - antioxidant index was calculated [16]. Also calculated in the mucosal homogenate of gingiva and cheeks were urease activity [17] and the level of lysozyme [11], determined by the ratio of oral dysbiosis - according to A.P. Levitskiy [18].

3. RESULTS AND DISCUSSION

Following the introduction of lipopolysaccharide (LPS), inflammation of oral (gingiva are cheek) mucosa was observed in the experimental animals, which caused a corresponding change in the biochemical parameters of inflammation (Table 1). The levels of malondialdehyde and elastase increased mainly in the gingival mucosa, . Previous application of the gel containing gold and silver nanoparticles had no significant effect on the elevated levels of elastase in the gingiva, which remains high, at the level of controls inflammation. Possibly, this is due to the very short duration (only 3 days) of application and to inadequate drug concentrations.

The occurrence of inflammation interferes with lipid peroxidation, which leads to increased levels of malondialdehyde in the gingiva. Previous application of gel with nanoparticles of gold and silver results in a decrease in malondialdehyde. At a silver concentration of 400 mg/g, the levels of malondialdehyde decreased up to almost normal ranges: up to 13.8 ± 1.0 mmole/kg, with normal levels of 13.4 ± 0.8 mg/kg.

Table 1. Influence of silica gel, containing nanoparticles of gold and silver, on the biochemical indices of the experimental inflammation of rat gingiva

Groups	Elastase, μKAT/kg	MDA, mmole/kg
1. Normal	41 ±6	13.4 ±0.8
2. LPS - control 1	62 ±2 p<0.01	20.7 ± 2.8 p<0.05
3. LPS+Lyzomucoid (LM) - control 2	57 ±2 p<0.05 pi>0.05	14.7 ±1.2 p>0.3 pi<0.05
4. LPS±LM±Au (5 nm, 500 mkg/g)	57 ±2 p<0.05 pi>0.05 P2=l	17.5 ±1.8 p<0.05 pi>0.3 p_2>0, 2
5. LPS±LM±Au (5 mkm, 400 mkg/g)	60 ±3 p<0.05 pi>0.5 p_2>0, 3	15.0 ±1.4 p>0.3 pi<0.05 p_2>0, 5
6. LPS±LM±Ag (5 mkm, 400 mkg/g)	59 ±3 p<0.05 pi>0.3 p_2>0, 3	16.3 ± 1.4 p>0.05 pi>0.05 p_2>0, 3
7. LPS±LM±Ag (10 mkm, 400 mkg/g)	59 ±2 p<0.05 pi>0.3 p_2>0, 3	13.8 ±1.0 p>0.5 pi<0.05 p_2>0, 3

Note: p - index of reliability of the difference from group 1, pi - index of reliability of the difference from group 2, p2 - index of reliability of the difference from group 3.

The inflammatory markers: malondialdehyde and elastase were increased in the mucosa of the cheek after introduction of lipopolysaccharide (LPS). Preliminary application of gel Lyzomucoid (LM) reduces to some extent the level of these biochemical parameters (Table 2). Application of gels with nanoparticles significantly enhance the anti-inflammatory effect of Lyzomucoid, but in neither group the level of inflammatory markers is below the normal range.

Table 2. Influence of silica gel, containing nanoparticles of gold and silver, on the biochemical indices of the experimental inflammation in rat cheek mucosa

Groups	Elastase, μKAT/kg	**MDA, mmole/kg**
1. Normal	51±3	13.1 ±1.2
2. LPS - control 1	69 ±2 p<0.01	21.3 ±1.9 p<0.05
3. LPS±Lyzomucoid (LM) - control 2	66 ±11 p>0.05 pi>0, 5	19.7 ±1.6 p<0.05 pi>0, 3
4. LPS±LM±Au (5 nm, 500 mkg/g)	61 ±4 p<0.05 pi<0.05 p_2>0, 3	19.0 ±2.1 p<0.05 pi>0.3 p_2>0, 5
5. LPS±LM±Au (5 mkm, 400 mkg/g)	62 ±2 p<0.05 pi<0.05 p_2>0, 3	18.0 ±1.7 p<0.05 pi>0.3 p_2>0, 5
6. LPS±LM±Ag (5 mkm, 400 mkg/g)	60 ±6 p>0.05 pi>0.05 p_2>0, 3	19.4 ±1.4 p<0.05 pi>0.3 p_2>0, 5
7. LPS±LM±Ag (10 mkm, 400 mkg/g)	57 ±6 p>0.3 pi<0.05 p_2>0, 05	17.6 ±1.4 p<0.05 pi>0.05 p_2>0, 3

Note: p - index of reliability of the difference from group 1, pi - index of reliability of the difference from group 2, p2 - index of reliability of the difference from group 3.

An index expressing the state of the antioxidant system is the level of catalase in the tissues of gingiva and oral mucosa. After lipopolysaccharide (LPS) application, the activity of catalase in the gingiva and cheek mucosa was significantly ($p < 0.05$) reduced, compared to normal values (Table 3). Application of the gel with lyzomucoid (LM) slightly increased the catalase activity, but this increase is statistically doubtful ($p > 0.05$). Application of gels with gold or silver nanoparticles enhances the activity of catalase in the tissues of gingiva and cheeks, however in neither group its level is below the normal level of activity. However, one can notice that the highest increase of activity occurred in the group of animals to which the gel with Lyzomucoid and nanoparticles of silver was applied.

Table 3. Influence of silica gel, containing nanoparticles of gold and silver, on the activity of catalase in the gingiva and cheek mucosa of rats under experimental inflammation

Groups	Gingiva	Cheek
1. Normal	7.85 ± 0.42	6.97 ± 0.47
2. LPS - control 1	6.03 ± 0.22 p<0.01	5.22 ± 0.52 p<0.05
3. LPS+Lyzomucoid (LM) – control 2	6.14 + 0.87 p<0.05 pi>0.5	6.00 ± 0.37 p>0.05 pi>0.05
4. LPS+LM+Au (5 nm, 500 mkg/g)	7.10 + 0.53 p>0.1 pi<0.05 p_2>0, 3	6.45 ± 0.29 p>0.1 pi<0.05 p_2>0, 05
5. LPS+LM+Au (5 mkm, 400 mkg/g)	7.83 ± 0.40 p>0.8 pi<0.01 p_2<0, 01	6.62 ± 0.33 p>0.3 pi<0.05 p_2>0, 2
6. LPS+LM+Ag (5 mkm, 400 mkg/g)	7.84 + 0.71 p>0.9 pi<0.01 p_2<0, 01	6.96 ± 0.32 p>0.9 pi<0.01 p_2>0, 05
7. LPS+LM+Ag (10 mkm, 400 mkg/g)	8.58 + 0.14 p>0.05 pi<0.01 p_2<0, 01	6.92 ± 0.44 p>0.8 pi<0.05 p_2>0, 05

Note: p - index of reliability of the difference from group 1, pi - index of reliability of the difference from group 2, p2 - index of reliability of the difference from group 3.

By calculating the ratio of catalase activity and the content of malondialdehyde (MDA), the prooxidant - antioxidant index (API), evidencing antioxidant and prooxidant balance of tissues, was determined. After application of lipopolysaccharide and inflammation in the mouth tissue, the antioxidant protection decreases, as evidenced by a sharp (almost 2-fold) reduction of the API index in cheek mucosa: up to 0.25 ± 0.1 *versus* 0.53 ± 0.14 under normal conditions.

Application of the Lyzomucoid (LM) gel slightly increases the level of the antioxidant - prooxidant index (API) to 0.30 ±0.1. Application of gels with gold and silver nanoparticles increases even more the antioxidant system of the mucosa cheek tissue: under the influence of the gel containing silver nanoparticles measuring 10 microns (group number 7), API increased up to 0.39 + 0.1.

Application of lipopolysaccharide on the oral mucosa of the mouth causes its inflammation, which leads to a significant (almost 3 times) increase in the activity of urease in gingiva (Table 4), causing a significant increase in the microbial contamination of gingival mucosa.

Previous applications of the Lyzomucoid gel significantly reduced urease activity. Addition of silver and gold nanoparticles to the gel cause a more pronounced decrease in urease activity,

which is most pronounced in the case of silica containing gold nanoparticles with a size of 5 microns and concentration of 400 mg/d (group number 5).

Table 4. Influence of silica gel, containing nanoparticles of gold and silver, on the activity of urease and lysozyme in the gingiva of rats under experimentally-induced inflammation

Groups	Urease, μKAT/kg	Lysozyme, units/kg
1. Normal	0.72 ±0.10	310±48
2. LPS - control 1	2.35 ±0.13 p<0.001	209 ± 20 p<0.05
3. LPS±Lyzomucoid (LM) - control 2	1.54 ±0.08 p<0.001 pi<0.01	227 ± 37 p>0.05 pi>0.3
4. LPS±LM±Au (5 nm, 500 mkg/g)	1.45 ±0.10 p<0.001 pi<0.001 p_2>0, 3	279 ±17 p>0.3 pi<0.05 p_2>0, 05
5. LPS±LM±Au (5 mkm, 400 mkg/g)	1.29 ±0.08 p<0.01 pi<0.01 p_2<0, 05	248 ± 28 p>0.05 pi>0.3 p_2>0, 3
6. LPS±LM±Ag (5 mkm, 400 mkg/g)	1.35 ±0.11 p<0.01 pi<0.01 p_2>0, 05	233 ± 26 p>0.05 pi>0.3 p_2>0, 5
7. LPS±LM±Ag (10 mkm, 400 mkg/g)	1.38 ±0.09 p<0.01 pi<0.01 p_2>0, 05	248 ± 22 p>0.05 pi>0.3 P2>0, 4

Note: p - index of reliability of the difference from group 1, pi - index of reliability of the difference from group 2, p2 - index of reliability of the difference from group 3.

After gingival disease (gingivitis) progress under the influence of lipopolysaccharide (LPS), the activity of lysozyme in gingival homogenate decreased (Table 4), indicating weakening of local nonspecific immunity [15]. Preliminary application of Lyzomucoid slightly increases the activity of lysozyme. Application of gels with nanoparticles of silver and gold significantly increases the activity of lysozyme, especially in the case of gel with 5 nm-sized gold nanoparticles and a concentration of 500 mg/d (group number 4).

Table 5 shows the results of urease and lysozyme in cheek mucosa of rats. Progress of inflammation leads to a significant (spread over more than 3.5-fold) increase in urease activity in cheek mucosa. Application of Lyzomucoid leads to decreased activity of urease, yet without reaching the normal range. Application of gels with nanoparticles of silver and gold causes a significant decrease in urease activity.

Table 5. Influence of silica gel, containing nanoparticles of gold and silver, on the activity of urease and lysozyme in the oral mucosa of rats under experimentally-induced inflammation

Groups	Urease activity, μKAT/kg	Lysozyme activity, units/ kg
1. Normal	0.43 ±0.12	310 ±34
2. LPS - control 1	1.41 ±0.05 P< 0.001	196 ±20 p<0.01
3. LPS±Lyzomucoid (LM) -control 2	1.21 ±0.05 p< 0.001 pi < 0..5	217 ±31 p > 0.05 pi > 0.3
4. LPS±LM±Au (5 nm, 500 mkg/g)	1.22 ±0.06 p< 0.001 pi < 0.05 p_2>0, 9	248 ± 22 p > 0.05 pi > 0.05 p_2>0, 3
5. LPS±LM±Au (5 mkm, 400 mkg/g)	1.35 ±0.12 p< 0.001 pi >0.3 p_2>0, 2	207 ±21 p < 0.05 pi > 0.4 p_2>0, 5
6. LPS±LM±Ag (5 mkm, 400 mkg/g)	1.09 ±0.12 p<0.01 pi < 0.05 p_2>0.3	206 ± 25 p < 0.05 pi > 0.4 p_2>0, 5
7. LPS+LM+Ag (10 mkm, 400 mkg/g)	1.09 ±0.11 p<0.01 pi < 0.05 p2>0.3	206 ± 25 p < 0.05 pi > 0.4 p2>0.5

Note: p - index of reliability of the difference from group 1, pi - index of reliability of the difference from group 2, p2 - index of reliability of the difference from group 3.

4.CONCLUSIONS

Due to experimental inflammation of the oral mucosa in the mouth, the activity of lysozyme in the mucosa cheek of animals is significantly reduced. Application of the Lyzomucoid gel slightly increases the activity of lysozyme. Application of gels with nanoparticles of silver and gold causes a significant increase in the activity of lysozyme, which appears as most pronounced after the application of a gel with 5 nm-sized gold nanoparticles and a concentration of 500 mg/d (group number 4).

Consequently, the use of gels with gold and silver nanoparticles has an antibiotic effect in the case of experimental inflammation of the mucosa of gingiva and cheeks.

Experimental studies show that drugs with nanoparticles of gold and silver enhance the anti-inflammatory effects of Lyzomucoid, this gain being possibly mediated through the inhibition of microorganisms in gingiva and cheek mucosa. The experimental data can serve as a basis for further development of drugs with nanoparticles of gold and silver for clinical use, in the treatment of periodontal diseases and oral mucosa.

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