INFLUENCE OF SILICAGEL CONTAINING GOLD AND SILVER NANOPARTICLES ON THE BIOCHEMICAL INDICES OF EXPERIMENTALLY-INDUCED INFLAMMATION OF ORAL MUCOSA

A. V. BORYSENKO[1], O. V. LYNOVYTSKA[1], O. B. TKACH[1]

1 „O. O. Bogomoleets" National Medical University, Kiev, Ukraine
Corresponding author: serhiydentist@mail.ru

Abstract

Aim. Experimental study on rat oral mucosa inflammation and on the influence of drugs containing gold and silver nanoparticles upon the biochemical markers of inflammation and disbiosis.

Materials and method. The study was conducted on 42 white rats, in which inflammation of the oral mucosa was experimentally induced by application of lipopolysaccharide (LPS). Preliminary applications, on the oral mucosa, of the gel containing gold and silver nanoparticles were made 2 days before experimental inflammation. The activity of the biochemical inflammation indices was determined in a gingiva and cheek mucosa homogenate.

Results. Application of lipopolysaccharide LPS on rat oral mucosa increased the elastase activity and MDA content, which indicates inflammation. Application of gels containing Lyzomucoid with nanoparticles of gold and silver reduces the biochemical inflammation markers, but only in the oral mucosa. Under the action of LPS, catalase activity significantly increased in both gingiva and oral mucosa. The gel containing Lyzomucoid preparations and gold and silver nanoparticles significantly increases the activity of catalase and the antioxidant-prooxidant index (IPA) in both tissues, while decreasing oral mucosa disbiosis.

Keywords: *oral cavity, lipopolysaccharide, nanoparticles, inflammation, anti-oxidants, disbiosis, gels, lyzomucoids*

1. INTRODUCTION

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

A large range of drugs: antiseptics, enzymes, antibiotics, keratoplasty, etc., are used for the treatment of inflammatory lesions of the oral mucosa, yet reports on their effectiveness are rather contradictory. In such treatments, the antibacterial agents occupy an important place. The main shortcoming in the treatment of mucous membrane lesions that opportunistic microflora quickly gets used to antibiotics, which leads to significant changes in microbiota, manifested as modifications in the ratio of normal and pathogenic organism representatives.

This situation calls for further improvement of antibacterial drugs with the creation of more efficient compounds, to which microflora will hardly get used. In this context, the utilization of drugs containing gold and silver nanoparticles looks very promising. Nanoparticles have unique chemical and biological properties, especially important in therapeutical and preventive actions. Previous studies demonstrated the significant antibacterial effect of drugs containing 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 finely silica, which greatly enhances their therapeutical effect [13]. Therefore, their beneficial effects and the regeneration processes they induce, as well as their anti-inflammatory effects and influence on the metabolic processes developed in the tissues of the oral mucosa should be carefully considered.

The aim of the present study was to evidence the effect of drugs containing gold and silver nanoparticles on the biochemical indices of

inflammation and disbiosis, in an experimental investigation of rat oral mucosa inflammation.

2. MATERIALS AND METHOD

The study was conducted on 42 white rats weighing 180 ± 10 g, whose oral mucosa was inflamed by the application of 0.5 ml gel containing intestinal endotoxin - lipopolysaccharide (LPS) [14, 15], the employed drug being Pirogenal (produced by Medgamal, Russia), lipopolysaccharide (LPS), obtained from *Salmonella typhi*. The gel was applied to the mucosa in a ratio of 75 mg/kg of body weight of animals, along a 24 h period.

2 days before, 0.5 g gel containing gold or silver nanoparticles per experimental animal (rat) was applied to the mucous membrane of the mouth. Silica gels with gold and silver nanoparticles were provided by the Department of Inorganic Chemistry, "Taras Shevchenko" National University of Kiev. The gels were prepared by mixing 1g of silica gel with silver and gold nanoparticles, Lyzomucoid (LM) and 95g of 3% sodium carboxymethylcellulose.

The animals were divided into 7 groups, with 6 subjects in each. In 5 groups of animals, application of gel containing silver and gold nanoparticles was carried out in an amount of 0.5ml per rat for 3 days, and 2 days prior to lipopolysaccharide (LPS) on the oral mucosa. Group 2, treated only with LPS gel, served as control group 1. Group 3 of animals, treated with Lyzomucoid and LPS gel, served as control group 2.

Animals were taken out of the experiment on the 4th day, after a 3 day- application of gels and 1 day-application of lipopolysaccharide (LPS), with thiopental anesthesia (20 mg/kg) by total bleeding. The mucous membranes of gums and cheeks were cut, specifically where the biochemical activity of inflammation indices - elastase activity, content of malondialdehyde, activity of the antioxidant enzyme catalase - were determined in the homogenate [16]. The ratio of catalase activity: content of malondialdehyde (MDA) prooxidant-antioxidant index was calculated [16], as well as the urease activity [17] and level of lysozyme [11] - determined by the degree of disbiosis, according to A.P.Levitskiy [18].

3. RESULTS AND DISCUSSION

After lipopolysaccharide (LPS) introduction onto the experimental animals, inflammation of the mucous membranes (gums and cheek mucosa) was observed, as well as a corresponding modification of the biochemical parameters of inflammation (Table 1). Particularly in the mucous membranes of gums, the levels of malondialdehyde and elastase increased. Previous application of gel with gold and silver nanoparticles will not significantly influence the high levels of elastase in gums, which remain high, at the level of controls' inflammation, which is probably due to the very short application time (only 3 days) and to the inadequate drug concentrations.

The presence of inflammation interferes with lipid peroxidation, which leads to increased malondialdehyde levels in gums. Previous application of gel with gold and silver nanoparticles decreases the malondialdehyde content. At a silver concentration of 400 mg/g, the malondialdehyde levels decreased up to almost normal values, namely 13.8 ± 1.0 mmole/kg, with normal levels of 13.4 ± 0.8 mg/kg.

Table 1 - Influence of silicagel containing gold and silver nanoparticles on the biochemical indices of the experimentally-induced inflammation of rat gums

Groups	Elastase, μKAT/kg	MDA, mmole/kg
1. Norm	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 p_1>0.05	14.7 ± 1.2 p>0.3 p_1<0.05
4. LPS+LM+Au (5 nm, 500 mkg/g)	57 ± 2 p<0.05 p_1>0.05 p_2=1	17.5 ± 1.8 p<0.05 p_1>0.3 p_2>0.2
5. LPS+LM+Au (5 mkm, 400 mkg/g)	60 ± 3 p<0.05 p_1>0.5 p_2>0.3	15.0 ± 1.4 p>0.3 p_1<0.05 p_2>0.5
6. LPS+LM+Ag (5 mkm, 400 mkg/g)	59 ± 3 p<0.05 p_1>0.3 p_2>0.3	16.3 ± 1.4 p>0.05 p_1>0.05 p_2>0.3
7. LPS+LM+Ag (10 mkm, 400 mkg/g)	59 ± 2 p<0.05 p_1>0.3 p_2>0.3	13.8 ± 1.0 p>0.5 p_1<0.05 p_2>0.3

Note: p - index of reliability of the difference from group 1, p1 - 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 cheek mucosa after lipopolysaccharide (LPS) application. Preliminary application of gel Lyzomucoid (LM) reduces to some extent the level of these biochemical parameters (Table 2). Application of gels with nanoparticles significantly enhances the anti-inflammatory effect of Lyzomucoid, however, in none of the groups the level of inflammatory markers is below the normal range.

Table 2 - Influence of silicagel containing gold and silver nanoparticles on the biochemical indices of experimentally-induced inflammation in the mucous membrane of rat cheeks

Groups	Elastase, μKAT/kg	MDA, mmole/kg
1. Norm	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 p_1>0.5	19.7 ± 1.6 p<0.05 p_1>0.3
4. LPS+LM+Au (5 nm, 500 mkg/g)	61 ± 4 p<0.05 p_1<0.05 p_2>0.3	19.0 ± 2.1 p<0.05 p_1>0.3 p_2>0.5
5. LPS+LM+Au (5 mkm, 400 mkg/g)	62 ± 2 p<0.05 p_1<0.05 p_2>0.3	18.0 ± 1.7 p<0.05 p_1>0.3 p_2>0.5
6. LPS+LM+Ag (5 mkm, 400 mkg/g)	60 ± 6 p>0.05 p_1>0.05 p_2>0.3	19.4 ± 1.4 p<0.05 p_1>0.3 p_2>0.5
7. LPS+LM+Ag (10 mkm, 400 mkg/g)	57 ± 6 p>0.3 p_1<0.05 p_2>0.05	17.6 ± 1.4 p<0.05 p_1>0.05 p_2>0.3

Note: p - index of reliability of the difference from group 1, p1 - index of reliability of the difference from group 2, p2 - index of reliability of the difference from group 3.

The index best expressing the condition of the antioxidant system is the level of catalase present in tissues of gums and oral mucosa. After lipopolysaccharide (LPS) application, the activity of catalase in gums and cheek mucosa was significantly ($p < 0.05$) reduced, compared to the normal one (Table 3). Application of the gel with lyzomucoid (LM) slightly increased catalase activity, which is statistically doubtful ($p > 0.05$). Applications of gels with nanoparticles of gold

or silver enhance the activity of catalase in the tissues of gums and cheeks, however in none of the groups the value recorded remained below the normal level of activity. The highest increase of activity occurred in the group of animals treated with gels of Lyzomucoid and silver nanoparticles.

Table 3 - Influence of silicagel containing gold and silver nanoparticles on the activity of catalase in rat gum and cheek mucosa with experimentally-induced inflammation

Groups	Gum	Cheek
1. Norm	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 p_1>0.5	6.00 ± 0.37 p>0.05 p_1>0.05
4. LPS+LM+Au (5 nm, 500 mkg/g)	7.10 ± 0.53 p>0.1 p_1<0.05 p_2>0.3	6.45 ± 0.29 p>0.1 p_1<0.05 p_2>0.05
5. LPS+LM+Au (5 mkm, 400 mkg/g)	7.83 ± 0.40 p>0.8 p_1<0.01 p_2<0.01	6.62 ± 0.33 p>0.3 p_1<0.05 p_2>0.2
6. LPS+LM+Ag (5 mkm, 400 mkg/g)	7.84 ± 0.71 p>0.9 p_1<0.01 p_2<0.01	6.96 ± 0.32 p>0.9 p_1<0.01 p_2>0.05
7. LPS+LM+Ag (10 mkm, 400 mkg/g)	8.58 ± 0.14 p>0.05 p_1<0.01 p_2<0.01	6.92 ± 0.44 p>0.8 p_1<0.05 p_2>0.05

Note: p - index of reliability of the difference from group 1, p1 - index of reliability of the difference from group 2, p2 - index of reliability of the difference from group 3.

Calculation of the ratio of catalase activity and of the malondialdehyde (MDA) content permitted determination of the prooxidant-antioxidant index (API), which evidences the antioxidant and prooxidant balance of tissues. After application of lipopolysaccharide and observed inflammation in the mouth, tissue 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 (normal value).

Applications with the Lyzomucoid (LM) gel slightly increase the level of the antioxidant-prooxidant index (API) to 0.30 ± 0.1, while application of gels with gold and silver nanoparticles increases considerably the antioxidant system of tissue mucosa cheek: under the influence of the gel containing silver nanoparticles, API increased to 0.39 ± 0.1(group number 7).

Application of lipopolysaccharide on the oral mucous membrane causes its inflammation, which leads to a significant (almost 3 times) increase of urease activity in gums (Table 4), indicating a significant increase in microbial contamination of mucous membranes gums. Previous applications with the Lyzomucoid gel significantly reduced urease activity. Addition of silver and gold nanoparticles to the gel leads to a more pronounced decrease in urease activity, which is the highest in the case of silica containing gold nanoparticles with a 5 micrometers size and a concentration of 400 mg/d (group number 5).

Table 4 - Influence of silicagel containing gold and silver nanoparticles on the activity of urease and lysozyme in rat gums with experimentally-induced inflammation

Groups	Urease, μKAT/kg	Lysozyme, units/kg
1. Norm	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 $p<0.001$ $p_1<0.01$	227 ± 37 $p>0.05$ $p_1>0.3$
4. LPS+LM+Au (5 nm, 500 mkg/g)	1.45 ± 0.10 $p<0.001$ $p_1<0.001$ $p_2>0.3$	279 ± 17 $p>0.3$ $p_1<0.05$ $p_2>0.05$
5. LPS+LM+Au (5 μm, 400 mkg/g)	1.29 ± 0.08 $p<0.01$ $p_1<0.01$ $p_2<0.05$	248 ± 28 $p>0.05$ $p_1>0.3$ $p_2>0.3$
6. LPS+LM+Ag (5μm, 400 mkg/g)	1.35 ± 0.11 $p<0.01$ $p_1<0.01$ $p_2>0.05$	233 ± 26 $p>0.05$ $p_1>0.3$ $p_2>0.5$
7. LPS+LM+Ag (10 μm, 400 mkg/g)	1.38 ± 0.09 $p<0.01$ $p_1<0.01$ $p_2>0.05$	248 ± 22 $p>0.05$ $p_1>0.3$ $p_2>0.4$

Note: p - index of reliability of the difference from group 1, p1 - index of reliability of the difference from group 2, p2 - index of reliability of the difference from group 3.

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

Table 5 shows the results of urease and lysozyme activity in cheek mucosa of rats. The progress of inflammation leads to a significant (more than 3.5-fold) increase of urease activity in the mucosa cheek. Applications of the Lyzomucoid gel reduce urease activity, yet not up to reaching normal values. Applications of gels with silver and gold nanoparticles reduce significantly urease activity.

Table 5 - Influence of silicagel containing gold and silver nanoparticles on the urease and lysozyme activity in rat oral mucosa under experimentally-induced inflammation

Groups	Urease activity, μKAT/kg	Lysozyme activity, units/kg
1. Norm	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 p_1 < 0.05	217 ± 31 p > 0.05 p_1 > 0.3
4. LPS+LM+Au (5 nm, 500 mkg/g)	1.22 ± 0.06 p < 0.001 p_1 < 0.05 p_2 > 0.9	248 ± 22 p > 0.05 p_1 > 0.05 p_2 > 0.3
5. LPS+LM+Au (5 mkm, 400 mkg/g)	1.35 ± 0.12 p < 0,001 p_1 >0.3 p_2 > 0.2	207 ± 21 p < 0.05 p_1 > 0.4 p_2 > 0.5
6. LPS+LM+Ag (5 mkm, 400 mkg/g)	1.09 ± 0.12 p < 0.01 p_1 < 0.05 p_2 > 0.3	206 ± 25 p < 0.05 p_1 > 0.4 p_2 > 0.5
7. LPS+LM+Ag (10 mkm, 400 mkg/g)	1.09 ± 0.11 p < 0.01 p_1 < 0.05 p_2 > 0.3	206 ± 25 p < 0.05 p_1 > 0.4 p_2 > 0.5

Note: p - index of reliability of the difference from group 1, p1 - 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 oral mucous membrane, the activity of lysozyme in rat mucosa cheek is significantly reduced. Applications with the Lyzomucoid gel slightly increase the activity of lysozyme, while applications of gels with silver and gold nanoparticles cause a significant increase in lysozyme activity, which is more pronounced after application of the gel with 5 nm in size gold nanoparticles and a concentration of 500 mg/d (group number 4).

Thus, the use of gels with gold and silver nanoparticles has an antibiotic effect in the case of experimentally-induced inflammation of the mucous membrane of gums and cheeks.

Experimental studies show that drugs containing gold and silver nanoparticles enhance the anti-inflammatory effects of Lyzomucoid, possibly through inhibition of microorganisms of gum and cheek mucosa. The experimental data here obtained 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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