Morphophysiological changes in the intact surface of rat skin under the application of silicon and gold nanoparticles

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Abstract. The effect of a course application of suspension of porous silicon and gold nanoparticles on the level of free radical oxidation and catalase activity in the skin homogenate was studied. MesoPSi samples were formed using the standard method of electrochemical etching of p-type c-Si wafers with a surface orientation of (100) and a specific resistance of 1-5 mOhm x cm in a solution of hydrofluoric acid and ethanol (HF (50%): C₂H₅OH = 1: 1) at an etching current density of 60 mA / cm² for 1 hour (45 minutes-hour). After this, mesoPSi films were separated from the silicon substrate by a short-term increase in the current density to 600 mA / cm². Aqueous suspensions of porous silicon nanoparticles with sizes of the order of 100 nm were obtained by mechanical grinding of mesoporous silicon films in water. Aqueous suspensions of gold nanoparticles with an average size of 30-50 nm were obtained by laser ablation of gold targets in deionized water. A suspension of silicon and gold nanoparticles in physiological saline at a concentration of 0.2 mg / ml was applied once a day in the form of a thin layer on a shaved area of the skin tissue but contributed to the activation of the rate of spontaneous lipid peroxidation.

1. Introduction

The use of nanoparticles in biology and medicine is increasing due to the fact that they increase the sensitivity and specificity of diagnostic tools and are used in targeted drug delivery. In the treatment of cancer, nanoparticles can reduce the dose of the active pharmacological agent without reducing the therapeutic effect. The study of the most common patterns of manifestation of biological and toxic effects of nanoparticles is the most important relevant task [1,2].

The aim of the work was to study the structural changes in the epidermis and dermis of rat skin when applying the solutions of gold and silicon nanoparticles on its intact surface, determine the level of free radical lipid oxidation and catalase activity in skin homogenates.
2. Materials and methods

In the experiment, 20 female outbred rats with an average weight of 210 g were used. The animals were 6 months old. Female rats were divided into three groups: 1) group I – control – animals whose physiological saline was applied to the skin; 2) group II - animals, on the skin of which nanosilicon particles were applied in aqueous suspension; 3) Group III - animals on the skin of which particles of nanogold were applied in aqueous suspension. MesoPSi samples were formed using the standard method of electrochemical etching of p-type c-Si wafers with a surface orientation of (100) and a resistivity of 1-5 mΩm x cm in a solution of hydrofluoric acid and ethanol (HF (50%): C₂H₅OH = 1: 1) at an etching current density of 60 mA / cm² for 1 hour (45 minutes-hour). After this, mesoPSi films were separated from the silicon substrate by a short-term increase in the current density to 600 mA / cm². Aqueous suspensions of porous silicon nanoparticles with sizes of the order of 100 nm were obtained by mechanical grinding of mesoporous silicon films in water [3]. Aqueous suspensions of gold nanoparticles with an average size of 30-50 nm were obtained by laser ablation of gold targets in deionized water.

A suspension of silicon and gold nanoparticles in saline at a concentration of 0.2 mg / ml was applied once a day in the form of a thin layer on a shaved area of animal skin (interscapular region) and left to dry completely. Applications were made daily for 10 days [4].

All animals were kept in standard vivarium conditions with free access to water and food. The killing of animals by decapitation was carried out after preliminary anesthesia with diethyl ether. When conducting all experiments, we took into account the requirements of the Directive of the European Parliament and the Council of the European Union for the Protection of Animals Used for Scientific Purposes (2010/63 / EU), Order of the Ministry of Health of the Russian Federation No. 199n dated 04/01/2016 “On Approval of the Laboratory Practice Rules” and the protocol Ethics Committee of FSBEI HE "Astrakhan State Medical University" of the Ministry of Health of Russia No. 8 dated November 24, 2015. A skin fragment (0.5x0.5 cm) was taken for morphological examination immediately after decapitation. Animals were decapitated under ether anesthesia. Skin samples were fixed in 10% formalin solution on phosphate buffer. The material was poured into paraffin. Slices 4-5 μm thick were made on a LabCut 4055 rotary microtome. For microscopy, the sections were stained with hematoxylin and eosin and enclosed in Canadian balsam. Structural and functional changes in the skin were evaluated. Microphotography of histological preparations was carried out on an Axio Scope 40 (Carl Zeiss) and Axio Star (Carl Zeiss) microscope with an integrated TV adapter and a Carl Zeiss Imager digital camera, A1, W-Pl 10x / 23 eyepiece, lenses: Achromplan 20x / 0.45 , 40x / 0.60, 63x / 0.80 and 100x / 1.25 oil. Morphometric analysis was performed using ImageJ software. Microphotographs (hematoxylin-eosin staining) of cross sections of the skin of animals from the control and experimental groups (5 sections from each animal) determined the thickness of the epidermis (μm), the thickness of the stratum corneum of the epidermis (μm), the thickness of the granular and basal layers of the epidermis (μm), the volume of basal keratinocytes (μm³) [6]. Statistical analysis of the results was carried out using the program Statistica 6.0.

A homogenate was prepared from a skin sample in which the level of free radical oxidation of lipids and catalase activity were measured [4]. The level of free radical oxidation was determined by the rate of spontaneous lipid peroxidation (spPOL) and the initial content of malondialdehyde (MDA) in the skin homogenate according to the method of I.D. Steel and T.G. Garishvili in the modification of E.A. Stroeva and V.G. Makarova [5]. Catalase activity in skin homogenate was determined by the method of M.A. Korolyuk et al., 1988 [7]. The data obtained were subject to statistical processing using Student's criterion.

3. Results and discussion

The results of morphological studies showed that the application of solutions of gold and silicon nanoparticles on the intact surface of the skin of rats leads to a number of characteristic changes in the structure of all layers of the epidermis and dermis (table 1, figure 1-4).
The thickness of the granular and basal layers of the epidermis, as well as the volume of basal keratinocytes, increased significantly when exposed to gold nanoparticles. The microstructure of skin preparations under the action of gold nanoparticles changed as follows: the stratum corneum of the epidermis was thinned, in comparison with control preparations, the scales were less tightly attached to the granular layer, the granular and basal layers of the epidermis were hypertrophic. Collagen fibers in the dermis are not damaged and have a correct orientation.

When exposed to a suspension of silicon nanoparticles, a significant thickening and compaction of the stratum corneum occurred. Cells resembling basal keratinocytes were present in the granular layer. Heteromorphy of the basal layer of the epidermis was observed. The pattern of collagen fibers in the dermis differed from that in the control. Some disorientation of the fibers was detected.
Figure 3. The microstructure of the skin of rats (gold nanoparticles group). Hematoxylin and eosin.

In general, changes in the epidermis and dermis indicate the absence of barrier properties of the skin under the action of silicon and gold nanoparticles. It can be concluded that keratinization is activated under the influence of gold nanoparticles. When exposed to silicon nanoparticles, the stratum corneum of the epidermis was compacted, desquamation of flakes from its surface was difficult.

| Morphometric indicators                      | Control   | Silicon nanoparticles | Gold nanoparticles |
|----------------------------------------------|-----------|-----------------------|--------------------|
| The thickness of the epidermis, mcm          | 65.3±6.81 | 69.8±7.82             | 84.2±7.78          |
| The thickness of the stratum corneum of the epidermis, mcm | 24.2±2.2 | 37.6±3.58             | 18.7±1.32*         |
| The thickness of the granular layer of the epidermis, mcm | 8.6±0.33 | 12.7±0.57             | 21.8±0.89*Δ       |
| The thickness of the basal layer of the epidermis, microns | 13.6±1.56 | 14.2±1.25             | 32.9±1.77*Δ       |
| The volume of basal keratinocytes, mcm³      | 152.3±12.25 | 161.7±14.28          | 176.2±11.23*      |

* the significance of differences between experimental groups compared to control; Δ significance of differences compared to silicon nanoparticles; θ significance of differences between experimental groups compared to gold nanoparticles
The level of free radical oxidation in the skin changed as follows (table. 2). The level of the initial MDA content in the experimental groups increased slightly compared to the same indicator in the control, while the SPPOL rate significantly increased in groups of animals that were exposed to nanostructured materials.

Table 2. The effect of silicon and gold nanoparticles on the level of free radical oxidation and catalase activity of the skin of female rats.

| Research indicators                                      | Control               | Silicon nanoparticles | Gold nanoparticles |
|----------------------------------------------------------|-----------------------|-----------------------|-------------------|
| The initial content of MDA in the skin tissue (nmol MDA / 500 mg of tissue) | 2.92±0.362            | 4.30±0.760            | 3.32±0.108       |
| The rate of spontaneous LPO (nmol MDA per 500 mg of tissue per 1 hour of incubation) | 8.70±0.880            | 12.97±1.821 *         | 21.40±5.742 *    |
| Catalase activity (mkat / l)                             | 89.9±10.00            | 77.7±8.00             | 78.0±14.94       |

* significance of differences between experimental groups compared to control

In this case, when applying silicon nanoparticles, this indicator increased by 1.47 times, while gold nanoparticles led to an increase in the rate of spontaneous LPO by 1.13 times relative to control animals (p≤0.05). The level of catalase activity remained virtually unchanged in comparison with the same indicator in control female rats (table 2). Thus, the studied silicon and gold nanoparticles did not change the initial MDA content and catalase activity in the skin tissue, but contributed to an increase in the rate of spontaneous lipid peroxidation.

4. Conclusion

Thus, no toxic or dystrophic changes in the epidermis were detected. At the morphological and biochemical level, the activation processes of keratinocyte metabolism under the influence of gold nanoparticles were found. Further studies using ultramicroscopic and histochemical methods are needed.
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