Contractile Reactions of Guinea Pig Airway Smooth Muscles in the Presence of Stannum Oxide Nanosized Particles

L. V. Kapilevich, T. N. Zaytseva*, A. V. Nosarev, B. G. Agev*, E. Yu. Dyakova, L. M. Ogorodova, A. A. Magaeva, O. G. Terecova**, and V. I. Itin****

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Contractile reactions of the guinea pig airway smooth muscles in the presence of stannum dioxide nanosized particles were studied. Contractile reactions to cholinergic and histaminergic stimulation were potentiated by inhalations of nanoparticle aerosol and by exposure of isolated smooth muscle segments to nanoparticle suspension.

Key Words: *smooth muscles; airways; nanoparticles*

Complex effects induced by exogenous nanoparticles and their interactions with biological objects have been described [2]. Nanomaterials enter the human body via several routes, most often through the lungs, from which they are rapidly transported with the blood stream into other vital organs [3]. Possible consequences of nanoparticle inhalation are changes in lung tissue and other organs resultant from the nanomaterial transport with the blood and changes in the levels of inflammation markers, active oxygen forms, *etc.* These changes and the mechanisms of their destructive effects are studied at the tissue and cell levels. Presumably, the changes in the organ functioning are explained by different composition of the nanomaterial [1,8].

We studied contractility of smooth muscle in guinea pig airways under conditions of exposure to nanosized particles of stannum dioxide.

Siberian State Medical University, Tomsk; *Institute of Atmospheric Optics, Tomsk Research Center, Siberian Division of the Russian Academy of Sciences; **Department of Structural Macrokinetics, Tomsk Research Center, Siberian Division of the Russian Academy of Sciences, Russia. ***Address for correspondence:*** zaytcevatn@yandex.ru. T. N. Zaytseva

MATERIALS AND METHODS

Annular smooth muscle segments of guinea pig airways (trachea and main bronchi) served as the object of our study. The animals were kept, fed, handled, and sacrificed in accordance with the "Regulations for Studies on Experimental Animals". The trachea and main bronchi were separated from the connective tissue, fat, and lung parenchyma in a cuvette with Krebs solution at ambient temperature. Contractile activity was studied on annular segments 3-4 mm long. The epithelium was removed mechanically by rotation of a wooded spatula in the segment lumen. Contractile reactions of the segments were studied by the mechanographic method. The segments were placed into a 4-ml chamber with warm aerated Krebs solution, fixed, stretched with a 500-mg load, and fixed to rods of mechanoelectric transducers. FT10G force pickup served as the mechanoelectric transducer, due to which the experiment was carried out under near-isometric conditions. The signal from the pickup was transferred to a 14-bite analog-to-digital converter (L791, L-Card) and recorded with a PC using LGraph-II software. The contractile responses to the test solutions were

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evaluated in percent of control contraction amplitude in response to hyperpotassium Krebs solution (40 mM KCl). Solutions of bioactive substances (carbacholine and histamine) in concentrations of 1 nM-10 μM served as the test solutions.

Light fraction of spherical particles 3-20 nm in size constituted 40 mas% in the sample of nanosized stannum dioxide particles, the rest constituted poorly aggregated particles 40-80 nm in size. Water solution of nanopowder at a concentration of 0.75 mg/ml was prepared for studies of inhalation exposure to the nanoparticles. A Musson-1M aerosol generator (Rotor Altai Engineering plant) was connected through its outlet to an isolated chamber in which the animal was placed. The generator produced aerosol (particle size 5 μ). An ELITE-801 air compressor was connected to the aerosol generator for creating directed aerosol flow (flow rate 1300 ml/min). Inhalations were carried out for 30 min daily for 4 days. Controls inhaled distilled water according to the same protocol. The effects of nanopowders added *in vitro* into the working chamber with smooth muscle segments were studied by adding the suspension of the nanoparticles at a concentration of 0.3 mg/ml.

The data were analyzed by Statistica 6.0 software (Statsoft) and presented as $M \pm m$. The distribution of the values was analyzed by the Kolmogorov–Smirnov normality test. The samples did not conform to the normal distribution law, and hence, statistical hypotheses were verified by nonparametric tests. The hypothesis on the homogeneity of two independent samples was tested by the Mann–Whitney *U* test. The homogeneity of paired or independent samples was tested by the Wilcoxon *T* test.

RESULTS

In experimental series I, the contractile reaction of airway smooth muscles in experimental animals inhaling a suspension of nanosized stannum oxide particles was compared to the reaction in the control animals inhaling distilled water according to the same protocol. The segments from all groups responded to carbacholine (1 nM-10 μM) by dose-dependent contractions. The segments from control animals developed a response reaching 87.71±4.29% in response to 10 μM carbacholine (EC_{50}=2.89±0.68 μM, n=12). The amplitude of contractions of airway segments of animals exposed to nanopowder inhalations surpassed the value in the controls and was 107.2±8.2% (n=12; p<0.05), the EC_{50} was lower than the control (1.4±0.3 μM).

Histamine (1 nM-10 μM) treatment of smooth muscle segments from control animals induced a contractile response (the maximum contraction in response to 10 μM histamine was 41.8±5.4%, EC_{50}=7.68±2.51 μM, n=13). Mechanical strain of airways segments from animals inhaling nanoparticle suspension significantly surpassed that of segments from control animals: 112.3±5.9% (n=11, p<0.05; Fig. 1). EC_{50} decreased to 2.4±0.9 μM.

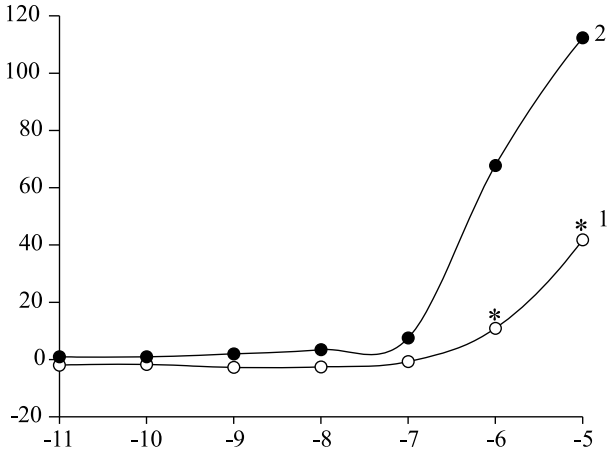

Fig. 1. Relationship between mechanical strain of intact smooth muscle segments and histamine concentration. *1*) segments of airways from control animals; *2*) segments of airways from animals after inhalation exposure to stannum dioxide. Ordinate: mechanical strain, in percent of control contraction amplitude in response to Krebs hyperpotassium solution; abscissa: histamine concentration decimal logarithm. *p<0.05 in comparison with the control.

In experimental series II, contractile reactions of airway smooth muscles in response to stannum dioxide nanoparticles were studied *in vitro*. The mechanical strain under conditions of carbacholine treatment of control segments reached 87.65±4.3%, EC_{50}=2.91±0.65 μM (n=13). After pretreatment with the nanopowder suspension, mechanical strain was 106.1±6.1%, EC_{50}=0.67±0.2 μM (n=12, p<0.05). The maximum strain of control segments under conditions of histamine treatment was 41.73±5.48%, EC_{50}=7.73±2.46 μM (n=13), that after pretreatment with nanosized particle suspension 53.7±9.6%, EC_{50}= 6.9±3 μM (n=11).

Hence, inhalation and *in vitro* exposure to nanosized particles led to potentiation of the contractile reactions of airway smooth muscles to histamine and carbacholine, manifesting by a greater amplitude of contractile responses and a lower concentration of the semi-maximum effect.

Changes in the contractile reactions in response to inhalation of nanoparticles can be caused by the formation of inflammation. Experimental studies indicate that nanoparticles can migrate in the bronchial tree epithelium and cause inflammatory reaction in the bronchial wall [5]. Changes in smooth muscle contractile activity under conditions of *in vitro* exposure to nanoparticles can be explained by reactions of the nanomaterial with cell membrane. The cell membrane

is not a barrier for some nanoparticles [5]. Presumably, the nanoparticles get into the cells by adhesion or diffusion, which is fraught with membrane injury. In addition, the molecules can neutralize cell surface charges [6]. Nanosized substances can react with endoplasmic proteins, modulate the cytoskeleton structure, disorder the actin element polymerization/depolymerization processes and the architecture of the microtubule network [4,7]. These disorders can modulate intracellular signaling and eventually modify smooth muscle contractility.

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