Environmental Toxicology and Pharmacology 50 (2017) 212–215

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Short communication

Silver nanoparticles alter the permeability of sheep pleura and of sheep and human pleural mesothelial cell monolayers

Zoi V. Arsenopoulou[a], Ioannis A. Taitzoglou[b], Paschalis-Adam Molyvdas[a], Konstantinos I. Gourgoulianis[c], Chrissi Hatzoglou[a], Sotirios G. Zarogiannis[a,*]

[a] Department of Physiology, Faculty of Medicine, University of Thessaly, BIOPOLIS, Larissa 41500, Greece
[b] Laboratory of Physiology, Faculty of Veterinary Medicine, Aristotle University, Thessaloniki, Greece
[c] Department of Respiratory Medicine, Faculty of Medicine, University of Thessaly, BIOPOLIS, Larissa 41500, Greece

ARTICLE INFO

Article history:
Received 28 November 2016
Received in revised form 16 January 2017
Accepted 4 February 2017
Available online 6 February 2017

Keywords:
Electrophysiology
Mesothelium
Nanotoxicology
Pleura
Silver nanoparticles
Ussing system

ABSTRACT

Nanoparticles have been implicated in the development of pleural effusions in exposed factory workers while in experimental animal studies it has been shown that they induce inflammation, fibrosis and carcinogenesis in the pleura. The scope of this study was to investigate the direct effects of silver nanoparticles exposure on the membrane permeability of sheep parietal pleura, of primary sheep pleural cell monolayers and on a human mesothelial cell line. Our findings suggest that acute (30 min) exposure increases the pleural permeability ex vivo, while longer (24 h) exposure in vivo leads to late decrease of the pleural cell monolayers permeability.

1. Introduction

Data obtained from animal studies have shown that inhaled carbon nanotubes reach the subpleural tissue in mice causing pleural inflammation and fibrosis, while abdominal injection of the same type of nanoparticles causes asbestos-like pathogenicity, such as peritoneal inflammation and granulomas (Bonner, 2010). In these studies a single inhalation and a single intraperitoneal dose were enough to elicit these effects. Moreover, intrapleural injection of silver nanotubes in mice demonstrated that nanofibers above 5 μm in length promote acute pleural inflammation and subsequent fibrosis (Schinwald et al., 2012).

Several clinical reports established a link between nanoparticle inhalation and pleural-related disease. A case series study reported that sustained inhalation of polyacrylate/nanosilica nanoparticles led to development of pleural effusion, inflammation, fibrosis and foreign body granulomas (Song et al., 2009). In a follow up analysis of the pleural fluid samples of these patients, nanoparticles of 2–20 nm size were detected, directly linking them with the pleural effusion development (Song et al., 2011). These clinical findings were also replicated in a rat model of polyacrylate/nanosilica nanoparticles intratracheal instillation, supporting the notion that inhaled nanoparticles can cause pleural effusion development along with inflammation and subsequent fibrosis (Zhu et al., 2016).

No studies up to now have investigated the direct effects of nanoparticles on the permeability of the pleural mesothelium. The aim of our study was to investigate whether silver nanoparticles alter the pleural transmesothelial resistance (R_{TM}), an inverse surrogate marker of pleural permeability (Vogiatzidis et al., 2006; Stefanidis et al., 2007; Zarogiannis et al., 2007), in ex vivo sheep pleural samples as well as in primary cultures of sheep mesothelial cells and a human mesothelial cell line (MeT-5A).

2. Materials and methods

2.1. Nanoparticles and reagents

Spherical silver nanoparticles (AgNPs) of 20 and 60 nm in diameter with known physicochemical characteristics were purchased from Sigma-Aldrich Chemie GmbH, Munich, Germany (#730793 and 730815 respectively).

* Corresponding author at: Environmental Pleural & Lung Diseases Group, Department of Physiology, Faculty of Medicine, University of Thessaly, 3 Panepistimiou Str BIOPOLIS, Larissa 41500, Greece.
E-mail address: szarog@med.uth.gr (S.G. Zarogiannis).

http://dx.doi.org/10.1016/j.etap.2017.02.009
1382-6689/

2.2. Sheep parietal pleura specimen collection

Intact sheets of sheep parietal pleura from the chest wall were stripped from adult sheep (males and females), immediately after the death of the animals (time of warm ischemia close to 0 min) from the slaughterhouse. Pieces of parietal pleura free from holes or adherent tissue were immediately placed in Phosphate-Buffered Saline (PBS) at 4 °C and transferred to the laboratory within 30 min. After arrival at the laboratory the pleural specimens were transferred to oxygenated Krebs-Ringer bicarbonate (KRB) solution, balanced at pH 7.4 and bubbled with 95%O_2-5%CO_2. PBS contained (in mM) 137 NaCl, 2.7 KCl, 10 Na_2HPO_4, 1.8 KH_2PO_4 supplemented with 1 $CaCl_2{\cdot}2H_2O$ and 0.5 $MgCl_2{\cdot}6H_2O$. KRB contained (in mM) 117.5 NaCl, 1.15 NaH_2PO_4, 24.99 $NaHCO_3$, 5.65 KCl, 1.18 $MgSO_4$, 2.52 $CaCl_2$, and 5.55 glucose. Pleural tissues were left in KRB for 30 min in order to equilibrate and then they were subjected to various treatments.

2.3. Incubation of sheep pleura with AgNPs and Ussing chamber experiments

Prior to each experiment AgNPs were vortexed at room temperature for 5 min and re-suspended in KRB in the final concentrations. Parietal pleura specimens were incubated either with 2 μg/mL 20 nm or 2 μg/mL 60 nm spherical AgNPs in KRB solution for 30 min, while corresponding controls were incubated with 0.2 mM sodium citrate-KRB solution (since purchased AgNPs were in aqueous sodium citrate solution). After the incubation with the experimental solutions, the tissues washed with KRB and mounted in Ussing chambers (K. Mussler Scientific Instruments, Aachen, Germany) with an opening surface area of 1 cm^2 for transmesothelial resistance (R_{TM}; Ω cm^2) measurements under open circuit conditions. All solutions were freshly prepared before each experiment, heated to 37 °C, and bubbled continuously with a 95% O_2/5% CO_2 gas mixture. Tissues were bathed with 4 ml KRB solution on each side of the membrane and were continuously oxygenated with 95%O_2-5%CO_2. After mounting the tissue to the Ussing System it equilibrated (for about 10–20 min) and then the R_{TM} measurement was registered.

2.4. Cell culture of sheep primary pleural mesothelial cells and the human pleural mesothelial MeT-5A cell line

Primary cultures of sheep pleural mesothelial cells were established by specimens of intact visceral sheep pleura obtained from the slaughterhouse immediately after the death of the animal. The procedure was performed under sterile conditions and pieces of approximately 2 cm^2 were washed with PBS and placed in transfer RPMI medium supplemented with 10% FBS, 5% penicillin – streptomycin and 1% L-Glutamine. The specimens were transferred to the laboratory on ice, washed with PBS and placed in sterile petri dishes coated with fibronectin (FN) solution (50 μg/mL FN from Calbiochem, USA) that were incised with a scalpel in 5 areas. The petri dishes were filled with 3 ml of supplemented RPMI medium (10% FBS, 1% penicillin – streptomycin and 1% L-Glutamine) and placed in a 5% CO_2 humidified incubator. The medium was changed the next day and then every third day. The specimens were discarded after 6 days and the adherent cells were cultured in supplemented RPMI medium. The human mesothelial cell line MeT-5A (benign transformed mesothelial cells) was also used for experiments. MeT-5A cells were grown under the same conditions. Primary cells and MeT-5A cells were plated onto tissue culture-treated polycarbonate filters (Transwell, 6.5 mm diameter, 0.4 μm diameter pores, 1.12 cm^2 area; Corning, Sigma) on Day 0 and grown according to manufacturer instructions. In each well 5×10^4 cells were seeded in 100 μl of media. Cells were maintained at 37 °C in a humidified 5% CO_2 incubator. Confluent monolayers were formed by Day 3.

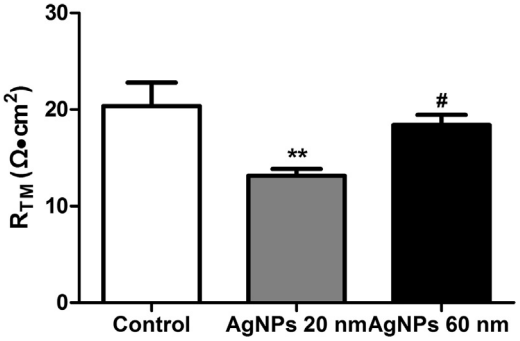

Fig. 1. Transmesothelial resistance (R_{TM}; Ω cm^2) of sheep parietal pleura specimens pre-incubated for 30 mins with 0.2 mM sodium citrate (Control; white bar; n = 14), 2 μg/mL 20 nm AgNPs (AgNPs 20 nm; grey bar; n = 16) and 2 μg/mL 60 nm AgNPs (AgNPs 20 nm; black bar; n = 15). $^{**}p < 0.01$ comparison vs. Control and $^{\#}p < 0.05$ comparison vs. AgNPs 20 nm.

2.5. Measurement of the effects of AgNPs on the R_{TM} of pleural mesothelial cell monolayers

R_{TM} (Ω cm^2) in the presence or absence of AgNPs as a function of exposure time (up to 24 h) were measured by the EVOMX Voltohmmeter with an STX2 electrode (World Precision Instruments, Inc), according to manufacturer instructions. All R_{TM} values were corrected for filter area and the background values were subtracted (values across blank filters). The monolayers were treated with 0.2 mM sodium citrate, 2 μg/mL 20 nm or 2 μg/mL 60 nm AgNPs in supplemented RPMI on Day 3 after plating. In one set of experiments, cells were exposed to AgNPs apically and in another set basolaterally. AgNPs were added at time 0 and effects of AgNPs on the R_{TM} was assessed at times 0, 1, 6 and 24 h.

2.6. Statistical analysis

Statistical analysis for was performed using GraphPad Prism 5. All data are expressed as mean ± S.E.M. The results presented are the means of the stated number of experiments in each case. Comparisons among three groups were done with One-way ANOVA and Tukey's multiple comparisons test for parametric data. Two-way ANOVA with Bonferroni post-test was used for comparison of two variables in more than three groups. Values of $p < 0.05$ were regarded as significant.

3. Results

3.1. Effects of AgNPs on sheep parietal pleura R_{TM}

The R_{TM} of control sheep parietal pleural specimens was 20.36 ± 2.44 Ω cm^2. Pre-incubation of parietal pleura specimens with 20 nm AgNPs for 30 mins resulted in a significant decrease in the R_{TM} as compared to control (13.13 ± 0.72 Ω cm^2; $p < 0.01$). Contrary to this finding the effect of 60 nm AgNPs pre-incubation did not differ significant from control (18.40 ± 1.04 Ω cm^2) while it was significantly higher compared to the 20 nm AgNPs ($p < 0.05$). These results are demonstrated in Fig. 1.