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Pilot study on in vitro silver nanoparticles permeation through meningeal membrane

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ABSTRACT

Silver nanoparticles (AgNPs) are used as a common ingredient in antiseptic sprays and mists; they can easily come into contact with the upper-airway mucosa. The intranasal pathway represents the only direct connection between the external environment and brain structures, which are generally considered to be well protected. Drugs absorption through this route has been widely studied, but toxicological knowledge is scant. The olfactory bundles are surrounded by meningeal sheets in their path from the nasal mucosa to the olfactory bulb. This study investigated the transmeningeal absorption of 19 nm AgNPs, using excised porcine meninges mounted on Franz diffusion cells in vitro. Two donor solutions were used: one containing AgNPs (0.5 g L^{-1}), and another containing only the water-soluble silver species derived from the ultrafiltration of the first one. Each experiment was carried out separately for 2 h. Results showed silver flux permeation through the meninges, with similar values in both experiments (0.78 ± 0.71 ng cm^{-2} h^{-1} and 0.73 ± 0.43 ng cm^{-2} h^{-1}, for AgNPs and Ag ions respectively, mean and SD). This study demonstrates that the meningeal barrier is permeable to silver and silver ions, when it is exposed to this metal in nanoparticular form; this might lead to neurotoxic and neurodegenerative effects, as recently shown by other studies. Silver nanoparticles are used by workers and consumers, and potential penetration through meningeal membrane needs to be considered and prevented when it is possible an inhalation exposure.

1. Background

Silver nanoparticles (AgNPs) are the main ingredient in many antibacterial sprays, which are commonly used on a wide variety of surfaces, in occupational and private settings. Despite European and American regulations [1], their use is often not supervised; in addition, there are no specific rules regarding their safe use in other regions of the world. Kim and his coworkers investigated some of these commercial products; a relevant risk of nanoparticles inhalation was found in some cases, as the margin of exposure was higher than the no-risk concern level of 1000 [2]. Furthermore, the use of spray containing AgNPs is even being recommended as a remedy for respiratory disorders such as asthma, which means that many patients could be exposed to considerable amounts of AgNPs through inhalation [3]. Although silver is generally well tolerated and non-toxic for humans, there are isolated reports of neurological, kidney and liver disorders as a result of its absorption in large quantities [4,5].

Inhalation exposure to NPs poses a well-known problem because of their possible translocation trough the alveolar-capillary barrier into the respiratory system, which can lead to a systemic uptake of xenobiotics. On the other hand, a possible direct absorption via the intranasal route – circumventing the very tight blood brain barrier (BBB) – is still an overlooked issue.

The central nervous system (CNS) is highly protected from xenobiotics penetration, due to the presence of barrier structures; however, there is a direct connection between the external environment and the front portions of the CNS via the olfactory nerve. The latter connects the olfactory bulb to the nasal cavity, passing through the openings in the cribriform plate of the ethmoid bone, which is covered by the dura mater. Along this route, the branches of the olfactory nerve are surrounded by extensions of the dura mater, which descend into the nose through these apertures [6].

This biological pathway has been extensively studied in recent decades, in association with drug administration in cognitive, neurodegenerative and psychiatric diseases, and also in some

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functional disorders of the CNS [7–12]. Studies have shown that nanoparticular drug administration conveys a 1.6–3.3 times higher substance concentration in all the compartments of the CNS (cerebral spinal fluid, olfactory bulb, olfactory tract, brain and cerebellum), compared to traditional drug administration [13].

To this day, the potential absorption of metal NPs by means of this mechanism has been poorly investgated; nevertheless, it represents a matter of concern, since the CNS may be exposed to the neurotoxic effects of xenobiotics in professional and/or environmental scenarios [14–16].

Absorption through this path can take place by intraneuronal passage (i.e. inside the sensory neurons) and also by extracellular passage, which consists of the transcellular path (through the sustentacolar supporting cells) and of the paracellular path (through the intercellular cleft) [17].

The aim of this study is to assess the potential permeability of the meningeal membranes that are involved in the extracellular pathway, whose penetration properties to silver nanoparticles (AgNPs, the most commonly used NPs) are still unknown.

The aim of this study is to assess the potential permeability of silver NPs through meningeal membranes that are the first barrier to CNS.

2. Material and methods

2.1. Chemicals

All the chemicals used in this study were analytical-grade. Sodium chloride, sodium hydrogen phosphate, potassium dihydrogen phosphate, nitric acid (69% v/v), hydrochloric acid (37% v/v) were purchased from Sigma Aldrich (Milan, Italy), while ammonium hydroxide (25%) was purchased from J.T. Baker (Milan Italy). Water reagent grade was produced with a Millipore purification pack system (milliQ water).

The physiological solution used as receptor fluid was prepared by dissolving 2.38 g of Na_2HPO_4, 0.19 g of KH_2PO_4 and 9 g of NaCl into 1 L of milliQ water (final pH = 7.35).

2.2. Silver nanoparticles characterization

Polyvinylpirrolidone-stabilized AgNPs (content of silver: 25% w/w, polymer 75%) were supplied by NanoAmor Materials Inc. (Houston, TX, USA). AgNPs from the same batch were characterized and used in previous permeation studies through ex vivo human skin [18] and through porcine oromucosal membrane [19].

Size and morphology of the AgNPs metal cores, while dispersed in physiological solution, were obtained by means of Transmission Electron Microscopy (EM208; Philips, Eindhoven, The Netherlands, operating at 200 kV) with an high definition acquisition system, based on a side-mounted TEM camera (OSIS Morada) and an iTEM software platform (Olympus Soft Imaging Solutions GmbH, Münster, Germany). The polymer coating composition was confirmed using a FT-IR Spectrum 100 (Perkin Elmer, Waltham, MA, USA; FT-IR, Fourier transform-infrared) fitted with an attenuated total reflection (ATR) (Ge/Ge) accessory [18].

The average values of the AgNPs size and polydispersity, defined as a relative width of the size distribution, were determined from Dynamic Light Scattering (DLS) measurements, using a Zeta sizer Nano Z (Malvern Instruments Ltd.) analyzer, applying a 633 nm laser with a 173° orientation with respect to the sample [19].

Zeta potential measurements were carried out using a Zetasizer-Nano ZS (Malvern). The zeta potential was calculated using Henry's equation [19].

2.3. Donor phases preparation

Two different donor phases were prepared immediately before the beginning of the experiments, in order to distinguish between the permeation of AgNPs and that of silver ions released from the NPs.

The first donor phase consisted of the AgNPs dispersion, which was prepared using 100 mg (with a 1:4 metal/polymer ratio) of AgNPs dispersed in 50 mL of physiological solution by sonication, in order to obtain a silver concentration of 0.50 g L^{-1}. Using the ultrafiltration technique, 5% of silver in ionized form was revealed in the nanoparticles water suspension. The presence of silver ions did not change significantly during the experiment, which lasted 2 h.

The second donor phase was prepared applying ultrafiltration to the first one, thus selecting the water-soluble silver species only. To separate the AgNPs from the aqueous solution, 4 mL of the AgNPs solution was ultrafiltered in a centrifuge at 5000 rpm for 30 min, using Amicon Ultra-4 centrifugal filters (10 KDa MWCO). Filtration was reiterated on five different aliquots, that were mixed for a total of 20 mL, in pursuance of an adequate volume for silver quantification analysis and permeation experiments.

In order to define the percentage of silver ions inside the AgNPs solution, the donor phases were analyzed by means of Inductively Coupled Plasma – Optical Emission Spectrometry (ICP–OES).

2.4. Preparation of meningeal membranes

Thanks to the high percentage of genomic and morpho-physiological similarities between pig and human [20,21], the pig model is commonly used in biomedical research studies, as well as in some cases of animal to human xenotransplantation.

Porcine meninges were collected from a slaughterhouse in Trieste, IT. The membranes were excised from the animal skull immediately after the slaughter. The pigs were up to 1 year old. The head was sawed in half along the cranium caudal line, in order to achieve access to the ventral surface of the skull region. The meningeal surface adherent to the skull was detached from the underlying bone with surgical forceps. For each animal two pieces (~5 cm diameter) of meninges (one from each side of the skull) were obtained. During the transportation to the laboratory, the tissue was stored at 4 °C; subsequently, it was stored in a refrigerator compartment at −80 °C, for a maximum time of 1 week.

Ex vivo studies have demonstrated that this cryopreservation temperature can adequately preserve neuronal cells viability and specific features [22,23].

The tissues were removed from the refrigerator and soaked in a physiological solution at room temperature for about 30 min before starting the permeation experiments.

Integrity of the membranes was checked before and after each experiment, using the following method: intact membranes were mounted on Franz Cells, and the donor chambers were filled with MilliQ water. The receiving chamber was monitored for presence of the solution for a period of 30 min, and if integrity was confirmed, the permeability experiment was commenced [24].

After the experiments the procedure was repeated, and if a leak of saline solution appeared in the receiving chamber, the membranes were discarded.

2.5. In vitro diffusion system

Meningeal permeation studies were performed using static Franz diffusion cells [25]. The receiver compartments had a mean volume of 14.0 mL and were maintained at 37 °C, in order to reproduce physiological conditions throughout the experiment, by means of thermostated water circulation in the jacket surrounding the cells. Salts concentration in the receiver fluids was approxi-