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In vitro meningeal permeation of $MnFe_2O_4$ nanoparticles

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ABSTRACT

$MnFe_2O_4$ nanoparticles (NPs) are commonly produced in some occupational settings and may reach high concentration in activities such as arc-welding or ferroalloy metallurgy. Manganese is an essential cofactor in enzyme activities but it has been demonstrated that long-term exposure to excessive levels can lead to "manganism", a neurodegenerative disease resembling Parkinson features.

Inhaled NPs deposit partially in pharynx and nasopharynx and may reach the central nervous system through the olfactory nerve, which is completely enveloped by the meningeal membranes throughout its course from the nasal cavity to the olfactory bulb or through the trigeminal nerves. This study investigated *in vitro* the transmeningeal absorption of 50 nm $MnFe_2O_4$NPs, using excised porcine meninges mounted on Franz diffusion cells. We tested two donor solutions: the first containing $MnFe_2O_4$NPs (2.0 g/L) and the second obtained by the ultrafiltration of the first one, in order to test only the NPs water soluble fraction. Each experiment was carried separately for 4 h. Results showed that no Mn flux permeation through the meninges occurred, since only trace of the metal was found in receivers solutions of cells exposed to MnFeNPs (5.5 ± 2.2 ng/cm^2), ultrafiltered solution (3.5 ± 1.5 ng/cm^2) and blank cells. (2.1 ± 0.6 ng/cm$^{2)}$ (mean and SE). Differences did not reach the statistical significance.

Our study shows – for the first time - that $MnFe_2O_4$NPs penetrate the meningeal membrane in a negligible amount, thus making unlikely the hypothesis of a transcellular and paracellular absorption through the olfactory nerve but not excluding the hypothesis of an active intraneuronal absorption.

1. Background

The wide production and use of nanomaterials occurred in recent decades motivates the interest of the scientific community investigating possible unknown nano-toxicological effects. Nanoparticles, indeed, are expected to exert more severe health effects than bulk counterparts [16] given their higher surface area to volume ratio [34,44], which means that a greater interaction with biological matrices could take place after all routes of exposure.

Manganese nanoparticles (MnNPs) could easily come in contact with human bodies either when intentionally produced (engineered NPs) or as a by-products of other manufacturing processes. In the first case, MnNPs can be found in the wastewater treatment, in catalysis processes, in batteries, sensors, supercapacitors production and also as contrast agent in MRI in experimental studies [12,15,39,42,54]. In the second case MnNPs can be found in arc-welding fumes, in ferroalloy metallurgy workplaces, iron and steel foundries and also as components of combustion emissions of power plant and coke oven [61]. In all the aforementioned scenarios the main exposure for workers is through inhalation pathway [24,25,56].

While lung absorption has been quite extensively studied, very little is known regarding a possible absorption through the olfactory route, which may take place after metal NPs mucosal deposition in the upper airways [46,57] or through the trigeminal pathway from the respiratory mucosa [32]. The central nervous system (CNS) has a direct connection with the external environment through the olfactory nerves. Throughout this route, from the upper portion of the nasal cavity to the olfactory bulb, passing through the ethmoidal cribriform lamina, the boundless of the olfactory nerve are enveloped by the meninges, whose permeation properties are still poorly known [2,49].

The mechanism of uptake and transport of metals into the olfactory system has been studied for different particles [52,58] and Mn was found to be taken up preferentially via the olfactory pathway.

In physiological conditions manganese is a cofactor for several

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enzymes required in neuronal and glial cell function [11,22], but an overexposure to this metal may cause a bioaccumulation in the caudate nucleus and hippocampus [31]. This leads to a progressive and permanent neuro-degenerative damage of the dopaminergic neurons, whose symptoms resemble Parkinson's disease [22]. The affected workers develop fatigue, headache, muscle cramps, loss of appetite, apathy, dystonia, hypokinesia, rigidity, and muscle tremors [9,10,21,24,25,48], which altogether define a condition called "manganism" [7,14,28,31,47]. The same symptoms have been described in rats exposed to MnO_2 NPs [37].

The aim of the present study was to assess the permeability properties of meningeal membranes, currently undefined, towards $MnFe_2O_4$NPs, which represent a common by products in the arch-welding fumes [5,29] and may exert chronic neurotoxic effects on humans. Experimental data on metal NPs are actually lacking and results may increase the knowledge in the field of occupational health and safety.

2. Material and methods

2.1. Chemicals

All chemicals used were of analytical grade. Sodium chloride, sodium hydrogen phosphate, potassium dihydrogen phosphate, hydrogen peroxide (30% w/w), and nitric acid (69% w/w) were purchased from Sigma Aldrich (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. $MnFe_2O_4$ nanoparticles characterization

Manganese ferrite nanopowder ($MnFe_2O_4$) used for the experiments was supplied by Sigma-Aldrich (Milano, IT), and was formed of 50 nm mean size nanoparticles, datum confirmed by Transmission Electron Microscopy (EM208; Philips, Eindhoven, The Netherlands, operating at 200 kV) analysis (Fig. 1).

2.3. Donor phases preparation

Two different donor phases were prepared just before the experiments in order to distinguish the permeation of MnFeNPs and that of Mn and Fe ions released from the NPs. The first donor phase consisted of the MnFeNPs dispersion, prepared using 100 mg of $MnFe_2O_4$ nanopowder dispersed by sonication in 50 mL of physiological solution (final concentration of 2.0 g/L).

The second donor phase was prepared by ultrafiltration of the first one, to obtain only the water-soluble species present in the first donor phase at the moment of the experiment. Four mL of the MnFeNPs solution were ultrafiltered in centrifuge at 5000 rpm for 30 min by means of Amicon Ultra-4 centrifugal filters (10 KDa MWCO) in order to separate the MnFeNPs from the aqueous solution. The filtration has been repeated on five different aliquots, that were mixed for a total of 20 mL, in order to obtain an adequate solution volume to perform metals quantification analysis by means of Inductively Coupled Plasma – Optical Emission Spectrometry (ICP–OES) and the permeation experiments.

2.4. Preparation of meningeal membranes

Porcine meninges were used given their high histological similarities with human ones [3,35]. The membranes were collected immediately after the slaughter of pigs up to 1 year old in a slaughterhouse in Trieste, IT. The tissue was excised from the ventral surface of the animal skull after the opening of the bone along the cranium caudal line. The membranes were detached from the underlying bone with surgical forceps. Two pieces of meninges (~5 cm diameter) for each animal were obtained. The tissue was stored at 4 °C during the transport to laboratory and then in freezer at −80 °C for a period of time up to, but not exceeding, 1 week. On the day of the experiments tissues were removed from the freezer and soaked in physiological solution at room temperature for about 30 min before starting the permeation experiments. The integrity of the membranes was tested before and after each experiment by filling the donor chamber with water MilliQ and by monitoring the presence of the solution in the receiving chamber for a period of 30 min [36].

2.5. In vitro diffusion system

The method of static Franz diffusion cells [26] was used to study the meningeal permeation properties. The donor solutions were different in exp. 1 and 2, in order to evaluate whether permeation capability was different between NPs and their ultrafiltered solution, while the receiving phase was composed in both cases of warm physiological solution, in order to reproduce the same conditions that can be found in the blood. To do this the receiver compartments were maintained at 37 °C by means of circulation of thermostated water in the jacket surrounding the cells throughout the experiment and the receiving solution was kept stirred in each receiving cell using a Teflon coated magnetic stirrer.

The sheets of meninges were clamped between the donor and the receptor compartment placing the dura mater side facing the donor compartment, with a final mean exposed area of the meningeal membranes of 3.29 cm^2. Experiments were carried out for 4 h and conducted as follows:

Experiment 1: At time 0, the exposure chambers of 4 Franz diffusion cells were filled with 1.5 mL of MnFeNPs suspension (0.91 mg cm^{-2}), corresponding to a concentration of 0.44 mg cm^{-2} of Fe e 0.22 mg cm^{-2} of Mn, in order to provide an infinite dose: the concentration in each cell was confirmed at the end of the experiments by means of inductively coupled plasma - optical emission spectroscopy (ICP-OES) analysis.

At selected intervals (20, 40, 60, 80, 100, 120, 180, 240 min) 1 mL of the receiving bathing solution was withdrawn and collected for the analysis, and immediately replaced with an equal volume of fresh made physiological solution. At the end of the experiment the meninges pieces were removed, washed three times with 3 mL of milli-Q water, and subsequently stored in the freezer at −25 °C together with meningeal bathing solutions and the donor solutions for the following analysis.

Experiment 2: The exposure chambers of 4 Franz diffusion cells were filled with 1.5 mL of the ultrafiltered solution, to verify differences between ions and nanoparticles permeation. The other test conditions were the same of the exp. 1.

Blanks: For each experiment two cells were added as blank. The experimental conditions were the same with the exception of the donor solution, which was composed only of physiological solution.

2.6. Meningeal digestion after the experiment

The meningeal membranes were cut into small pieces after being dried for 2 h at room temperature and weighed. The sections were subsequently put into glass tubes with 10 mL of HNO_3 and 1.0 mL of H_2O_2 for digestion. The resulting solutions were heated at 80 °C for 8 h and then a final volume of 10.0 mL was restored with MilliQ water. The analyzes were performed by means of ICP-OES.

2.7. Quantitative analysis

An Inductive Coupled Plasma Mass Spectrometer NEXION 350D (Perkin Elmer; Waltham, MA, USA) with an integrated auto sampler,