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Influence of a low dose of silver nanoparticles on cerebral myelin and behavior of adult rats

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ABSTRACT

Nanoscale particles have large surface to volume ratio that significantly enhances their chemical and biological reactivity. Although general toxicity of nano silver (nanoAg) has been intensively studied in both *in vitro* and *in vivo* models, its neurotoxic effects are poorly known, especially those of low-dose exposure. In the present study we assess whether oral administration of nanoAg influences behavior of exposed rats and induces changes in cerebral myelin. We examine the effect of prolonged exposure of adult rats to small (10 nm) citrate-stabilized nanoAg particles at a low dose of 0.2 mg/kg b.w. (as opposed to the ionic silver) in a comprehensive behavioral analysis. Myelin ultrastructure and the expression of myelin-specific proteins are also investigated. The present study reveals slight differences with respect to behavioral effects of Ag^+- but not nanoAg-treated rats. A weak depressive effect and hyperalgesia were observed after Ag^+ exposure whereas administration of nanoAg was found to specifically increase body weight and body temperature of animals. Both nanoAg and Ag^+ induce morphological disturbances in myelin sheaths and alter the expression of myelin-specific proteins CNP, MAG and MOG. These results suggest that the CNS may be a target of low-level toxicity of nanoAg.

1. Introduction

Nanosilver (nanoAg), one of the most commonly used metal-derived nanomaterials, has broad-spectrum antibacterial and antifungal properties. These qualities have promoted its use in many life domains. NanoAg is currently used in a wide array of commercial goods, biomedical products (such as medical implants, catheters and wound dressings), as well as in food production and bioengineering (Marambio-Jones and Hoek, 2010; Cushen et al., 2014). One of the main aims of nanomedicine is to enhance drug availability within the central nervous system (CNS) by providing a mechanism for delivery of drugs past the blood-brainbarrier (BBB) to increase therapeutic efficacy (Sharma et al., 2013; Chaloupka et al., 2010). NanoAg is regarded as a nanoparticulate drug-carrier system for delivery of drugs to CNS (Leite et al., 2015).

The increasing production and use of engineered nanomaterials may inevitably lead to contamination of the environment and thus warrants risk assessment. On the other hand, medical applications also carry potential risks of toxic effects in humans. Hence, there is a need for research on low-level toxicity of nanoAg.

A number of studies, both *in vitro* and *in vivo*, have indicated toxic properties of nanoAg in a wide range of concentrations and sizes. Smaller nanoAg particles have been shown to be more active in exerting toxicological responses, inducing organ toxicity and inflammatory responses after repeated oral administration (Park et al., 2010). Current findings indicate that nanoAg may easily cross the BBB and accumulate in the rodent brain after oral or intravenous administration (Kim et al., 2008; Tang et al., 2010). Using an *in vitro* model, Trickler et al. (2010) showed that by releasing proinflammatory cytokines, nanoAg may induce inflammation in rat brain microvessels, which additionally enhances the breakdown of the BBB. Dysfunction of the BBB facilitates further penetration of nanoAg into brain tissue which causes synaptic (Skalska et al., 2015) and neuronal (Tang et al., 2009) degeneration.

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When administered intranasally, nanoAg alters spatial reference memory (Davenport et al., 2015) and locomotor activity in neonatal rats (Yin et al., 2015). NanoAg has also been reported to affect concentrations of the neurotransmitters dopamine and 5-hydroxytryptamine (5-HT) in the rat brain (Hadrup et al., 2012) and to impair spatial cognition in rats *via* their impact on hippocampal synaptic plasticity (Liu et al., 2012).

Except for a few neurotoxicological reports, the exact influence of nanoAg on CNS processes and underlying mechanism(s) of the action are not well understood. It has been reported that larger nanoAg particles (50–60 nm) in a relatively high dose of 50 mg/kg b.w. may cause myelin damage as reflected by myelin basic protein (MBP) immunostaining (Sharma et al., 2013). However, extensive research on the toxic impacts on myelin in adult organisms as well as a comprehensive assessment of behavioral effects have not yet been conducted.

Thus, the aim of the present study was to investigate whether prolonged oral administration of a low dose (0.2 mg/kg b.w.) of nanoAg (10 nm) or ionic silver (Ag^+) influences the behavior of rats and affects myelin ultrastructure and expression of myelin-specific proteins. A wide range of behavioral assessments was applied to assess the effects of nanoAg. Measurements of body weight and body temperature and tests of locomotor activity, motor coordination, nociceptive reaction, memory performance and anxiety-like behavior were performed. These measurements provide generally-accepted assessments of behavior in investigations of bioactivity of new compounds. Studies on behavioral impacts were combined with examination of expression of several myelin-specific proteins: myelin oligodendrocyte glycoprotein (MOG), myelin-associated glycoprotein (MAG) and 2′-3′-cyclic nucleotide 3′-phosphodiesterase (CNP). Since proteins are integral components of myelin membranes and contribute to their structure and stability, ultrastructural observations were also performed using transmission electron microscopy (TEM).

2. Materials and methods

2.1. Chemicals

Most of the chemicals used in the experiments, including nanoAg particles, were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). All reagents were of analytical grade.

NanoAg particles (10 ± 4 nm in diameter) (CAS No.730785) are defined by the manufacturer as a colloidal solution stabilized in sodium citrate to prevent sedimentation and to maintain the dispersed state. Characterization of the degree of dispersion and size distribution of the nanoAg particles was accomplished by transmission electron microscopy (JEM-1200EX, JEOL) using a digital camera MORADA and iTEM 1233 software (Olympus Soft Imaging Solutions GmbH, Germany) according to a standard method developed for non-biological preparations as described previously (Skalska et al., 2015).

2.2. Animals and experimental design of silver exposure

Male Wistar rats weighing 140–160 g obtained from the Farm of Labolatory Animals, Z. Lipiec, Brwinow, Poland were used throughout the study. All animals were caged in groups of six and maintained on a 12 h light-dark cycle (lights on at 6:00 h) at a controlled temperature (21 °C). During the experiments, the animals were allowed free access to drinking water and a standard laboratory diet known as the Murigan diet (Agropol, Motycz, Poland). All experiments were carried out according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and the European Community Directive for the Care and Use of Laboratory Animals, as of 24 November 1986 (86/609/EEC), and were approved by the Local Ethic Committee (35/2014).

Rats were randomly divided into three groups, each consisting of 12 animals and representing: (i) a negative control group (treated with saline), (ii) a nanoAg-treated group and (iii) an ionic silver-treated group (silver citrate, Ag^+). Solutions of nanoAg stabilized in citrate buffer or silver citrate were administered *via* a gastric tube in a dose of 0.2 mg/kg b.w. per day for 14 days (0.02 mg nanoAg or Ag^+/mL). The rats of the control groups received the same dose of saline.

During the experiment, body temperature and body weight were assessed. Body temperature in normothermic rats was measured at the animal's rectum with a thermistor thermometer twice daily on the 1st, 8th and 14th days of the experiment to check the acute effects of administration (the second measurement was taken 1 h after administration). Body temperature changes (Δt) were calculated according to the formula: $\Delta t = t2 - t1$, where $t1$ and $t2$, were the pre-substance and post-substance body temperature, respectively (Vogel, 2008).

Simultaneously, on the 1st, 8th and 14th day of the experiment, each animal was weighed.

2.3. Performance of behavioral tests

Behavioral tests were initiated after the final treatment on day 14. Locomotor activity and motor coordination were measured on the 15th day of the experiment. Anxiety and memory tests were carried out on the 16th day. On the last day of the study and before decapitation, the nociception reaction was assessed.

2.3.1. Locomotor activity test

Locomotor activity of individual rats was recorded using a photocell device (plexiglass boxes – square cages, 60 cm on each side; Porfex, Bialystok, Poland) in a sound-attenuated experimental room, under moderate illumination (10 lx). Ambulatory activity (distance traveled) was measured by two rows of infrared light-sensitivity photocells, installed along the long axis, 45 and 100 mm above the floor. Total horizontal activity (the distance traveled in meters) was recorded for a 30-min period (Koltunowska et al., 2013).

2.3.2. Rotarod performance test

To evaluate the muscle – relaxant or ataxic effects, rats were tested on a rotating rod (rotarod) apparatus (Multiserv, Lublin, Poland). Two days before the experiment, the animals were trained on a rotarod with a 6 cm diameter and 50 cm length, subdivided into four areas by disks 25 cm in diameter, at a constant rotating speed of 9 rpm. The rats that did not fall off the rotarod within 1 min were assigned the maximum score of 60 s (Kotlinska et al., 2012).

2.3.3. Novel object recognition test (NOR)

The apparatus included a square open box, made of plexiglass (63 cm long × 44.5 cm high × 44 cm wide) and illuminated by a lamp (light intensity – 10 lx), suspended 50 cm above the box. The objects to be discriminated were constructed of either of wood or plastic were in the shape of a block and a ball and were too heavy to be displaced by the animals.

The object recognition test was performed as described elsewhere (Bertaina-Anglade et al., 2006). The day before the test, each rat was placed in the empty box for 2 min to become acclimatized to the environment. On the day of the experiment, the animals were subjected to two trials, spaced apart by a 1-h interval. The first trial (acquisition trial, T1) lasted 5 min and the second trial (test trail, T2) was 3 min long. During T1, the apparatus contained two identical objects (wooden blocks), placed in two opposite