Toxicology Letters 214 (2012) 251–258

Contents lists available at SciVerse ScienceDirect

Toxicology Letters

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

Silver nanoparticles effects on epididymal sperm in rats

Joanna Gromadzka-Ostrowska[a,*], Katarzyna Dziendzikowska[a], Anna Lankoff[b,c], Małgorzata Dobrzyńska[d], Christine Instanes[e], Gunnar Brunborg[e], Aneta Gajowik[d], Joanna Radzikowska[d], Maria Wojewódzka[b], Marcin Kruszewski[b,f]

[a] Warsaw University of Life Sciences-SGGW, Nowoursynowska 159C, 02-776 Warsaw, Poland
[b] Institute of Nuclear Chemistry & Technology, Dorodna 16, 03-195 Warsaw, Poland
[c] The Jan Kochanowski University of Humanities and Sciences, Świetokrzyska 15, 25-410 Kielce, Poland
[d] National Institute of Public Health – National Institute of Hygiene, Chocimska 24, 00-791 Warsaw, Poland
[e] Norwegian Institute of Public Health, Lovisenberggata 6, 0456 Oslo, Norway
[f] Institute of Rural Health, ul. Jaczewskiego 2, 20-090 Lublin, Poland

HIGHLIGHTS

- We tested the effects of different sizes and doses of nanosilver (AgNPs) on male rats.
- Sperm count, germ cell DNA damage and seminiferous tubule morphometry were measured.
- Intravenously administered low dose of small AgNPs have a toxic effect on germ cells.
- They also caused changes in sperm counts.
- Obtained results suggested a genotoxic effect of low dose small size AgNPs.

ARTICLE INFO

Article history:
Received 18 May 2012
Received in revised form 29 August 2012
Accepted 31 August 2012
Available online 7 September 2012

Keywords:
DNA damage
Intravenous injection
Epididymis

ABSTRACT

The motivation of our study was to examine the acute effects of intravenously administered a single bolus dose of silver nanoparticles (AgNPs) on rat spermatogenesis and seminiferous tubules morphology. In the treated rats compared to the vehicle treated control animals, the experiments revealed a size-dependent (20 nm and 200 nm), dose-dependent (5 and 10 mg/kg body mass) and time-dependent (24 h, 7 and 28 days) decrease the epididymal sperm count measured by histological methods. In parallel AgNPs injection increased the level of DNA damage in germ cells, as measured by alkaline comet assay. Histological examination of the testes showed change in the testes seminiferous tubule morphometry in 200 nm Ag NPs treated rats. No change of body weight, adipose tissue distribution and the frequency of abnormal spermatozoa was observed. Twenty nanometers AgNP appeared to be more toxic than 200 nm ones.

1. Introduction

In the recent years nanomaterials had found a number of applications in everyday life. Despite of many benefits that nanomaterials, including nanoparticles (NPs), bring to the society, e.g. in drug delivery systems, medical devices, food products, cosmetics, etc. their increasing usage raises concern about the consequences and health threats that it might bring to humans. The risk of dermal, inhalation and ingestion exposure to NPs exist at many stages of NPs life cycle (Johnston et al., 2010). Moreover, NPs might be also intentionally introduced to the human body during medical procedures. Once entered the body, NPs are absorbed and translocated to different organs through the circulatory and lymphatic system (Panyala et al., 2008). Despite increasing knowledge of potential adverse health effects caused by exposure to NPs, available toxicological data in vivo are still not sufficient and contradictory in some cases.

Among various nanomaterials, silver NPs (AgNPs) became the NPs commonly used in material science, chemistry and consumer products due to their antibacterial and catalytic properties (Marambio-Jones and Hoek, 2010; Silvestry-Rodriguez et al., 2007). AgNPs are also used in medicine, as one of the constituent elements of dental alloys, catheters, implant surfaces and for treating of wounds and burns related infections, as well as in drug delivery in cancer and retinal therapies (Kalishwaralal et al., 2010; Samuel and

* Corresponding author at: Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Sciences-SGGW, Nowoursynowska 159C, 02-776 Warsaw, Poland. Tel.: +48 22 59 37 028; fax: +48 22 59 37 031.
E-mail address: joanna_gromadzka_ostrowska@sggw.pl (J. Gromadzka-Ostrowska).

0378-4274/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxlet.2012.08.028

Guggenbichler, 2004). Despite of their widespread use, the influence of AgNPs on human health and the mechanism of their action are not fully understood. Therefore, it is particularly important to investigate their potential toxicity in living organisms, especially in mammals, in order to provide a reliable human risk assessment (Panyala et al., 2008).

Most of the current research examining AgNPs toxicity is focused on in vitro models. These studies suggest that AgNPs have a potential cytotoxic and proinflammatory effect, caused mainly by increased production of reactive oxygen species, release of cytokines and upregulation of heat shock proteins (Kiruba et al., 2010; Kruszewski et al., 2011; Sung et al., 2009). Some reports show also that AgNPs induce apoptosis and necrosis, cause DNA damage and disturb cell cycle progression (Lankoff et al., 2012; Wijnhoven et al., 2009). Moreover, some studies reported that AgNPs induce changes in the gene expression, especially in the oxidative stress related genes (Bouwmeester et al., 2011). The in vivo studies confirmed the in vitro results and showed also that AgNPs can be accumulated and redistributed between various organs, leading to decreased body weight, changes in blood biochemical parameters and inflammation (Sung et al., 2009; Kim et al., 2010; Dziendzikowska et al., 2012).

Although it became clear that AgNPs may constitute a potential hazard to the human health and environment, their effect on the reproductive system, especially on the male reproductive functions, is still obscure. In this study we attempted to determine in vivo the size-, dose- and time-dependent effects of AgNPs on epididymal sperm count, frequency of abnormal spermatozoa and germ cell DNA damage in sperm cells and also testes seminiferous tubule morphometry.

2. Materials and methods

All materials and chemicals used were purchased from Sigma (USA) otherwise indicated.

2.1. Nanoparticle preparation

Spherical AgNPs with a nominal diameter of 20 ± 5 nm and 200 ± 50 nm were purchased from PlasmaChem (Berlin, Germany). AgNPs stock solutions were prepared by dispersion of 5 mg of nanoparticles in 800 μl of 0.9% NaCl solution. The AgNPs dispersions were sonicated for 3 min on ice using a probe sonicator (Branson, Danbury, Connecticut, USA) with 420 J total ultrasound energy. One hundred microliters of 15% bovine serum albumin and 100 μl of a 10× concentrated phosphate buffered saline solution were added immediately after sonication. Fresh stock solutions were prepared before each experiment.

2.2. Characterization of nanoparticles

A detailed characteristic of AgNPs was published previously (Lankoff et al., 2012).

Briefly, nanoparticle size and surface charge were measured by dynamic light scattering (DLS) on the Zeta-sizer Nano ZS (Malvern, Malvern Hills, UK). Stock solutions were diluted 1:4 with distilled water and measured in triplicates with 20 sub-runs. Zeta potential (ZP) measurements were performed at 25 °C in a folded capillary cell at 150 V and M3-PALS detection using non-invasive backscatter at 173° with an Avalanche photodiode, Q.E. > 50% at 633 nm (Malvern, Malvern Hills, UK). Samples were diluted 1:8 with distilled water and measured in triplicate with 20 sub-runs. ZP was calculated using the Smoluchowski limit for the Henry equation with a setting calculated for practical use ($f(ka) = 1.5$).

Shape and size of tested AgNPs was determined using a scanning electron microscope (SEM) type DSM 942 (Carl Zeiss, Göttingen, Germany) in the secondary electron mode. SEM worked with parameters: high voltage HV 2 kV, working distance WD = 4.7 mm. Stock solutions of nanoparticles were diluted 1:10 with distilled water and deposited on the microscopic holders. After the evaporation of the solvent (24 h), the samples were coated with a thin layer of Au (about 10 nm) using a vacuum evaporator (JEE-4X, JEOL, Tokyo, Japan) to protect the sample from heat destruction and to keep real parameters of the observed details. It operated with ultimate pressure 6×10^{-4} Pa with rotating and tilting specimen stage. The images were collected at 100,000× magnification.

2.3. Experimental design

This study was performed on 96 adult 14 weeks old male Wistar rats (strain: Wistar Cmd: WI(WU)) delivered by Mossakowski Medical Research Center, Polish Academy of Science (registered by District Veterinary Authorities No. 14313512). Animals were kept in polyurethane individual cages under stable environmental conditions (temperature 23 °C, humidity 60%, photoperiod L:D 12:12) with free access to food (Sniff® R/M-H, Sniff, Spezialitäten Gmbh G/M-H, Soest, Germany) and fresh water. All procedures were performed according to the Polish biological tests regulations and approved by 3rd Local Ethical Commission in Warsaw, Poland. After one week of acclimatization, animals were weighed (initial mean body weight 308.1 ± 22.4 g) and divided into 4 groups of 24 rats per group. Animals were injected (tail vein) with a single dose (5 mg/kg or 10 mg/kg) of 20 nm AgNPs (groups Ag I and Ag II, respectively) or with 5 mg/kg of 200 nm AgNPs (group Ag III). Sham-exposed rats were injected with 0.9% NaCl solution. Animals body weight gain was measured every week. Animals from the experimental and control groups were anesthetized by isoflurane inhalation and bleed by cardiac puncture 24 h, 7 days and 28 days after injection. Twenty eight days is the time period of half spermatogenesis and two cycles of the seminiferous epithelium and two cycles passage of germ cell through the epididymis (Kolasa et al., 2009; Bilińska et al., 2006). Sampling was performed within the first 2 h of light phase of the diurnal cycle. Testes and epididymides were removed and weighed. The left testicle from each rat was fixed in Bouin's solution (Sigma–Aldrich, Germany) for 24 h (Kula et al., 2001). The right testicle was frozen in liquid nitrogen and stored at −80 °C until analysis.

2.4. Epididymal sperm count and sperm morphology

The left epididymis was macerated and minced in 0.8 ml of 1% trisodium citrate solution for 7–8 min, then more solution was added (up to total amount 8 ml) and mixed for about 1 min. The sperm suspension was diluted 1:1 in 10% buffered formalin. Spermatozoa were counted using improved Neubauer haemocytometer (Harrison and Moore, 1980). The content of the right epididymis was used for estimation of the frequency of morphologically abnormal spermatozoa, according to the procedure described by Wyrobek and Bruce (1975). Cells were suspended in 0.9% NaCl and mixed. Smears were prepared on microscope slides, air dried overnight and stained with eosin Y. Then 1000 spermatozoa per rat were analyzed using a light microscope and all abnormalities were recorded.

2.5. Sperm cell DNA damage

The analysis of DNA damage was performed using the comet assay. A slice of the right testis from each animal was placed in RPMI1640 medium and minced, until single cells remained in the suspension. The alkaline comet assay was performed according to Kruszewski et al. (1995), with small modifications. In brief, 3 μl of the cell suspension were mixed in an Eppendorf tube with 75 μl of a low melting point agarose (LMPA). The final cell density was approximately 1.5×10^5/ml. The cell suspension was then pour on slides previously covered with a normal melting point agarose (NMPA). After agarose solidification at 4 °C another layer of LMPA was added and allowed to solidify. The slides were immersed in a lysing solution at 4 °C for at least 24 h, drained, placed in a gel electrophoresis tank and incubated in an alkaline ($pH \geq 13$) electrophoresis solution for 20 min to allow DNA unwinding. Electrophoresis was conducted for 20 min at 4 °C (electrophoresis parameters: 24 V; 0.6 V/cm; 300 mA). After neutralization, the slides were stained with ethidium bromide and examined using a fluorescence microscope. Images of 100 randomly selected cells from each animal were recorded and analyzed using the CASP image-analysis software (Końca et al., 2003). The % DNA in tail was chosen as a parameter for further analysis of DNA damage.

2.6. Histological investigation

After 24 h in Bouin's solution (Kula et al., 2001), the left testicle was processed through graded alcohol (POCH, Warsaw, Poland) and embedded in paraffin using standard histological techniques. The paraffin blocks were cut in slices (5 μm) and mounted on silanized microscope slides. Sections (3 per animal) were stained using a standard periodic acid-Schiff (PAS) method. The condition of the seminiferous tubules (space between them) and the continuity of germ epithelium were examined under a light microscope (Olympus, Warsaw, Poland) at a magnification of 4×. The spermatogenesis was estimated from the measurements of seminiferous tubules sections under the light microscope equipped with CCD camera and "F-View Soft Imaging System" (Olympus, Warsaw, Poland). The measurements were done using CellP computer software (MicroImage 4.0 Olympus Software). For each animal 25 tubules with visible circular cross section were randomly chosen and spermatids in the maturation phase of spermiogenesis were counted. In addition, for each tubule the following parameters were determined: area, circumference and diameter.