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Progressive effects of silver nanoparticles on hormonal regulation of reproduction in male rats

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ABSTRACT

The growing use of silver nanoparticles (AgNPs) in various applications, including consumer, agriculture and medicine products, has raised many concerns about the potential risks of nanoparticles (NPs) to human health and the environment. An increasing body of evidence suggests that AgNPs may have adverse effects of humans, thus the aim of this study was to investigate the effects of AgNPs on the male reproductive system.
Silver particles (20 nm AgNPs (groups Ag I and Ag II) and 200 nm Ag sub-micron particles (SPs) (group Ag III)) were administered intravenously to male Wistar rats at a dose of 5 (groups Ag I and Ag III) or 10 (group Ag II) mg/kg of body weight. The biological material was sampled 24 h, 7 days and 28 days after injection.
The obtained results revealed that the AgNPs had altered the luteinising hormone concentration in the plasma and the sex hormone concentration in the plasma and testes. Plasma and intratesticular levels of testosterone and dihydrotestosterone were significantly decreased both 7 and 28 days after treatment. No change in the prolactin and sex hormone-binding globulin concentration was observed. Exposure of the animals to AgNPs resulted in a considerable decrease in 5α-reductase type 1 and the aromatase protein level in the testis. Additionally, expression analysis of genes involved in steroidogenesis and the steroids metabolism revealed significant down-regulation of *Star*, *Cyp11a1*, *Hsd3b1*, *Hsd17b3* and *Srd5a1* mRNAs in AgNPs/AgSPs-exposed animals.
The present study demonstrates the potential adverse effect on the hormonal regulation of the male reproductive function following AgNP/AgSP administration, in particular alterations of the sex steroid balance and expression of genes involved in steroidogenesis and the steroids metabolism.

1. Introduction

The use of nano-sized materials in industrial, consumer and household products as well as in medical devices has dramatically increased over the past decades due to their advantageous and unique properties, including their high reactivity, mechanical strength, and high electrical and thermal conductivity (Stone et al., 2010). Silver nanoparticles (AgNPs) are one of the most commonly used nanomaterials due to their strong antibacterial, antiviral and antifungal properties. In particular, AgNPs have been used in cosmetics, food packaging, dietary supplements, cloths, toys and medical products such as bandages, wound and burn dressing, surgical instruments and implants (Reidy et al., 2013). However, such widespread use of AgNPs might lead to unforeseen biological effects in the environment and in living organisms, including potential harmful responses (Kruszewski et al., 2011).

Abbreviations: AgNPs, silver nanoparticles; AgPs, silver particles; AgSPs, silver sub-micron particles; Aro, aromatase; b.w., body weight; BSA, bovine serum albumin; *Cyp11a1*, cytochrome P450, family 11, subfamily A, polypeptide 1; *Cyp19a1*, cytochrome P450, family 19, subfamily A, polypeptide 1 (aromatase); DHT, dihydrotestosterone; E2, 17β-estradiol; *Gapdh*, glyceraldehyde-3-phosphate dehydrogenase; 25-HC, 25-hydroxycholesterol; *Hsd3b1*, hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1; *Hsd17b3*, hydroxysteroid (17-beta) dehydrogenase 3; *i.v.*, intravenously; 7-KC, 7-ketocholesterol; LH, luteinising hormone; NPs, nanoparticles; NOAEL, no observable adverse effect level; PBS, phosphate-buffered saline; PRL, prolactin; SHBG, sex hormone-binding globulin; SPs, sub-micron particles; *Srd5a1*, steroid-5-alpha reductase, alpha polypeptide 1 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 1); *Star*, Steroidogenic Acute Regulatory Protein; T, testosterone; TiO_2NPs, titanium dioxide nanoparticles; LDL, low-density lipoprotein.

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Experiments conducted on animal models have shown the nanomaterials' ability to cross the blood-testis barrier and to accumulate in testicular somatic and germinal cells (Morishita et al., 2012; Garcia et al., 2014). Morishita et al. (2012) reported significant accumulation of nanosilica in Sertoli cells and spermatocytes, including in the nuclei of spermatocytes after intravenous (*i.v.*) administration. Komatsu et al. (2008) observed titanium dioxide NP (TiO_2NP) accumulation in Leydig cells as agglomerates dispersed throughout the cytoplasm. They also observed reduced vitality and cell proliferation as well as altered expression of genes involved in response to oxidative stress and steroidogenesis in mouse Leydig cells *in vitro*. The published data also suggested that AgNPs could enter the testis and accumulate there (Kim et al., 2010; Garcia et al., 2014). Moreover, Asare et al. (2012) suggested that 20 nm AgNPs and 200 nm silver submicron-particles (AgSPs) are more cytotoxic and cytostatic as compared to 21 nm TiO_2NPs in established and primary testicular cell lines. The authors reported apoptosis and necrosis, increased yield of DNA strand breaks and decreased proliferation of cells exposed to AgNPs in both a concentration- and time-dependent manner. An *in vivo* animal model study also revealed that AgNPs caused genotoxicity and DNA damage in the testis (Asare et al., 2016).

Exposure to AgNPs can occur *via* ingestion, injection and inhalation, thus the testes can also be one of the organs harmed by the potential toxic effects of AgNPs (Kruszewski et al., 2011). However, the reproductive and developmental toxicity of NPs has been studied only in a few *in vivo* models (Ema et al., 2010). Takeda et al. (2009) detected aggregates of TiO_2NPs in the offspring of subcutaneously injected pregnant mice. *In utero* exposure to TiO_2NPs led to morphological changes in seminiferous tubules, significantly lowering daily sperm production and epididymal sperm motility. Similar effects were observed in rats after AgNP administration in adulthood (Miresmaeili et al., 2013). The authors suggested that AgNPs exerted acute effects on spermatogenesis, spermatogenic cell counts and acrosome reaction in sperm cells. Our recent studies also revealed a decrease in the sperm count, higher frequency of abnormal spermatozoa in epididymal semen, significantly increased DNA damage in germ cells and disorganisation of seminiferous tubule morphology as a result of *i.v.* AgNP administration in rats (Gromadzka-Ostrowska et al., 2012).

Many conditions and factors associated with male infertility can be attributed to hormonal disorders. A key role in the regulation of gonadal morphology and spermatogenesis is played by the concerted action of androgens, particularly testosterone (T) and dihydrotestosterone (DHT). Testosterone, whose biosynthesis is under the control of luteinising hormone (LH), is essential for the maturation of male germ cells and sperm production (Walker, 2009; Pantalone and Faiman, 2012). It regulates male fertility, either directly or through its metabolites: 17β-estradiol (E2) and DHT, the latter having the highest biological activity among all androgens (Mawhinney and Mariotti, 2013). As the activity of DHT is complementary to T action, 5α-reductase conversion of T to DHT is essential for physiological development and functioning of the male reproductive system (Auchus, 2004).

A recent study has suggested that nanomaterials represent evolving types of novel endocrine disruptors that may cause reproductive toxicity (Iavicoli et al., 2013). Although the toxicity of AgNPs has recently received much attention, its effect on the mammalian male reproduction system is still obscure. We hypothesised that the observed decrease in the sperm count caused by AgNP exposure may be associated with hormonal disorders and perturbation of steroidogenesis. Thus the aim of the present study was to examine the adverse effects of an intravenously (*i.v.*) administered single dose of AgNPs or AgSPs on male rats' reproductive function regulation, sex steroid metabolism and steroidogenesis-related gene expression. In addition, a mechanism of AgNP-induced hormonal distortions in rat testes is proposed.

2. Materials and methods

2.1. Nanoparticle characterisation

Spherical Ag particles (AgPs) of nominal diameter 20 ± 5 nm and 200 ± 50 nm (referred to here as Ag 20 and Ag 200, respectively) were delivered by PlasmaChem (Berlin, Germany). Nanoparticle hydrodynamic size and zeta potential were measured by dynamic light scattering (DLS) on a Zeta-sizer Nano ZS (Malvern, Malvern Hills, UK) (Table 1). A detailed characteristic of the AgNPs used here can be found in Gromadzka-Ostrowska et al. (2012). Particle stock solutions were prepared by dispersion of 5 mg of nanoparticles (NPs) in 800 μL of 0.9% NaCl solution. The AgPs were sonicated on ice for 3 min using a probe sonicator (Branson, Danbury, CT, USA) with 420 J/m^3 total ultrasound energy. Subsequently, 100 μL of 10× concentrated phosphate-buffered saline (PBS) and 100 μL of 15% bovine serum albumin (BSA) were added promptly after sonication, after Lankoff et al. (2012). The particles were prepared immediately before being administered to the animals in order to avoid their agglomeration in the suspension.

2.2. Experimental design

The study was conducted on fourteen-week-old male Wistar rats (strain: Wistar Cmd: WI(WU)) obtained from the Mossakowski Medical Research Centre, Polish Academy of Sciences (Warsaw, Poland, registered by District Veterinary Authorities No. 14313512) which were allowed to acclimatise for 10 days before the experiment began. During the experiment the animals were held in individual polyurethane cages. Household conditions were as follows: temperature 23 °C, 60% relative humidity and a 12-hour light-dark cycle. The animals had free access to water and commercial feed (Sniff® Spezialitäten GmbH R/M-H, Soest, Germany). The study was approved by the 3rd Local Ethical Commission in Warsaw (Poland) and performed in compliance with the applicable provisions of the national law.

After acclimatisation, the rats ($n = 93$) were divided into 4 groups: Ag I ($n = 24$), Ag II (n = 24), Ag III (n = 24) and the control ($n = 21$). The initial body weight of the animals was 308 ± 22 g. 20 nm AgNPs in two different doses (5 mg/kg body weight (b.w.) (Ag I group) and 10 mg/kg b.w. (Ag II group)) and 200 nm AgSPs at a dose of 5 mg/kg b.w. (Ag III group) were administered *i.v.* to the treated animals as a single bolus to the tail vein in order to evaluate the role of both size and dose of AgPs on reproduction regulation in male rats. Martínez-Gutierrez et al. (2012) showed the highest level of antimicrobial effectiveness of AgNPs in a size range between 20 nm and 25 nm, therefore, selection of 20 nm AgNPs allowed to assess the impact of AgNPs with high application potential. On the other hand, 200 nm Ag particles are beyond the nanoscale (usually up to 100 nm), thus they might serve as a reference material when size dependence is taken into consideration. Rats from the control group were injected with 0.9% NaCl solution. The animal's body mass gain and its activity were observed during the study. The results of our study suggest the good condition of all animals regardless of the experimental group. We found no effects of AgP exposure on the overall health of the animals, including body

Table 1
Characterisation of Ag 20 and Ag 200 particles in water after dispersion (mean ± SD), modified from Lankoff et al. (2012).

	Particles	
Indices	Ag 20	Ag 200
Particle nominal size [nm]	20 ± 5.0	200 ± 50.0
Dynamic light scattering (DLS)[a] [nm]	197.4 ± 2.7	422.4 ± 6.3
Polydispersity index	0.295	0.328
Zeta potential [mV]	−33.6	−37.5

[a] Dynamic light scattering (DLS) measurements are the average of at least 3 runs, each containing 20 sub-measurements