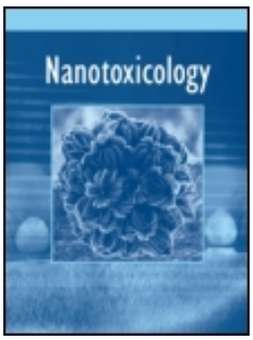

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Are nanoparticles potential male reproductive toxicants? A literature review

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Abstract
The rapid advancement of nanotechnology has prompted the need to investigate the health effects of nanoparticles and nanomaterials. The current focus of health and safety investigations has targeted routes of exposure and potential deposition, translocation, and adverse effects in primary and major secondary target organs. Few studies have looked at deposition in reproductive organs, and even fewer have assessed potential adverse effects on germline cells. This review summarizes the current published research on deposition/translocation of nanoparticles to the testes and male germline cells, and the potential cytotoxic effects. Six research articles were identified. Three articles pertained to deposition/translocation of nanoparticles in the testes, two pertained to cytotoxicity of nanoparticles on male germline cells, and one study assessed deposition and bioaccumulation of nanoparticles in the testes, and potential for adverse reproductive outcomes in successive offspring. While research into the potential reproductive toxicity of nanoparticles is still in its infancy, the identified research suggests that nanoparticles cross the blood testes barrier and deposit in the testes, and that there is potential for adverse effects on sperm cells. Suggestions for future research strategies are outlined.

Keywords: *Nanoparticles, nanomaterials, reproductive toxicity, testes, germline cells, sperm*

Introduction

The health effects of nanoparticles/nanomaterials are of great importance for nanotechnology to move forth in a responsible and sustainable manner. The current literature on the health and safety of nanoparticles/ nanomaterials is primarily focused on routes of exposures and potential deposition, translocation, and adverse effects in primary and major secondary target organs (lungs, skin, liver, spleen, brain, kidneys). Few studies have looked at deposition of nanoparticles in reproductive organs such as ovaries and testes, and even fewer have assessed potential adverse effects on germline cells *in vitro* or *in vivo*. The toxicology literature that is present on this subject is primarily focused in the biomedical and pharmacology literature and pertains to developing non-toxic and nonviral carriers for safe and effective drug delivery for cancer treatments, gene therapy, and cellular therapy.

While there is speculation that nanoparticles might pose potential reproductive harm, there has been little substantive evidence to support or refute these concerns. The objective of this review is to evaluate what is currently known about deposition/translocation of nanoparticles to the testes and male germline cells, as well as the potential cytotoxic effects of nanoparticles on male germline cells and/or sperm.

Scope of nanoparticle use

Nanoparticles are defined as particles ranging in size from 1 nm to 100 nm in diameter. Novel mechanical, electrical, optical, thermal, and magnetic properties have been discovered for various nanoparticles. For example, copper nanoparticles smaller than 50 nm are considered extremely hard materials that do not exhibit the same malleability and ductility as bulk copper, and ferroelectric materials smaller than 10 nm can switch their magnetism direction using thermal energy. Current applications for these materials are widespread throughout the various sciences, and include but are not limited to research in areas of biology, pharmacology, medicine, chemistry, physics, material science, and engineering. A multitude of consumer products from cosmetics and sunscreens to stain-resistant pants and sporting gear contain nanomaterials. The scope of use of nanoparticles has

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not even begun to scratch the surface of its projected potential. In coming years, nanomedicines are going to become available allowing for targeted gene therapies and cancer treatments. Potential exposure to nanoparticles is far reaching and has the future potential to include biomedical scientists, manufacturing workers, and everyday consumers. The human health effects and exposure limits of these materials will only be found through careful research into the health and safety of these new materials.

Routes of exposure and translocation/deposition of nanoparticles

Nanoparticles primarily enter the body through inhalation or dermal contact, and secondarily by injection or ingestion if nanoparticles are introduced into medicines or foods. Current animal research shows that after exposure to nanoparticles, by inhalation, ingestion, or injection, deposition can occur throughout the body and particles can translocate from primary exposure sites like the lungs and skin to secondary sites such as the liver, spleen, kidneys, muscle, brain, ovaries, and testes. Traces of these nanoparticles have also been found in the blood and urine of these animals shortly after exposure.

Most notable about nanoparticle deposition and translocation is their ability to cross the blood brain barrier as well as the blood testes barrier (Araujo et al. 1999; Kim et al. 2006). These barriers are highly selective and are important in protecting the brain and gametes from potential harmful exposures. While blood barrier permeability has been shown, it remains unclear what specific properties of nanoparticles (i.e., size, shape, polarity, etc.) allow for passage through these barriers.

Materials and methods

Search design

The scientific literature published in the last 25 years was searched. This period was chosen to capture all literature published on the subject of nanotechnology, although the literature on deposition/translocation and toxicity of nanoparticles has only recently (within the last 5 years) begun to develop. Only in the past year has research been published on the potential toxicity of nanoparticles on sperm *in vivo*.

Identification of studies

Studies were primarily identified using Medline, Web of Science, and EMBASE, and secondarily by hand-search of cited references from retrieved articles. Searches were conducted using the following key words: Nanoparticle, nanoparticles, and nanomaterials in combination with any of the following terms: deposition, translocation, toxicity, reproductive toxicity, sperm, spermatogonia, germline, testis, and testes. Articles were limited to those published in English.

Eligibility criteria

All articles pertaining to deposition/translocation of nanoparticles to testes/ male germline cells and/or cytotoxicity of nanoparticles on male germline cells and/or sperm were identified and reviewed.

Results

Eight research articles published within the last 25 years were identified as pertaining to deposition/ translocation of nanoparticles to the testes/male germline cells and/or cytotoxicity of nanoparticles on male germline cells and/or sperm. One identified study (Chen et al. 2006), which focused on acute toxicological effects of copper nanoparticles in mice after oral gavage, was excluded from this review because although the testes were weighed during analysis to determine potential deposition, no deposition data were presented in the research article. Four studies pertained to deposition/translocation of nanoparticles (Araujo et al. 1999; Chen et al. 2003; Kim et al. 2006; Kashiwada 2006), while two studies pertained to cytotoxicity on male germline cells or sperm (Braydich-Stolle et al. 2005; Makhluf et al. 2006). One study (Xue et al. 2005) assessed deposition and bioaccumulation of nanoparticles in the testes, as well as reproductive outcomes in successive offspring over one and a half years.

Deposition/translocation of nanoparticles

Objective of studies. Table I details the five studies evaluating the deposition/translocation of nanoparticles to the testes and male germline cells. The five studies identified focused on either pharmacology applications of nanoparticles or environmental exposures to nanoparticles. The main objective of three of the four pharmacology studies (Chen et al. 2003; Kim et al. 2006; Xue et al. 2005) was to determine toxicity of a coated nanoparticle to determine if they had good biological characteristics to act as promising vectors for gene transfer and gene/drug delivery, while the main objective of one of the pharmacological studies (Araujo et al. 1999) was to assess different solution suspensions for oral administration of nanoparticles and subsequent uptake in various

Table I. Translocation/deposition of nanoparticles.

Author (year)	Objective	Animal/cell line	Nanoparticle, size, concentration	Exposure	Findings
Chen et al. (2003)	Look at potential transfection method using SNAP and described the biodistribution of silica Nanoparticles in the various organs, tissues, cells.	Kunming white mice 17–21 g	SNAP coated with either sodium iodide or sodium chloride	Injection of SNAP suspension into either the abdominal cavity or the tail vein of the mice at different concentrations ranging from 0–225.0 mg/kg body weight. 96 h after administration mice were sacrificed	Deposition of SNAP in testicles, gland and interstitial cells of testes
Kim et al. (2006)	Used magnetic NPs to evaluate *in vivo* biological distribution and potential toxicity (looked at BBB function) to determine if good biological characteristics to act as promising vectors for gene transfer and gene/drug delivery.	Male IRC mice (6 weeks old)	MNPs@SiO2 (RITC), water-soluble, 50 nm thickness	Concentrations used: 100, 50, 25, 10 mg/kg administered intraperitoneally for 4 weeks. Mice were sacrificed after designated time periods (every week) to analyze tissue distribution and perform toxicity studies	No toxic effects. NPs cross blood testes barrier. More research needed.
Kashiwada (2006)	Assess environmental fate/ emerging environmental concerns for engineered NP being released into the environment and the distribution of NP in eggs and bodies of See-through Medaka (fish).	See-through Medaka	39.4 nm diameter-sized fluorescent particles at 1 mg/l (2.78% solids-latex solution)	Exposed See-through Medaka eggs to fluorescent nanoparticles	NP detected in testes but no statistically significant p-value (0.052) $n=8$
Xue et al. (2005)	Investigate the features and toxicology of modified SiNPs	Kunming white mice 17–21 g	SiNP, 40 nm, 0–225 mmg/kg	Injected into abdominal cavity or tail vein 4x in 2 months	Small amount of SinP found in testicle, gland and interstitial cells of testes. No pathological change in cell nucleus of these cells. No acute toxicity or mortality even at high concentrations. No apparent abnormalities found in subsequent generations.
Araujo et al. (1999)	Investigate uptake of PMMA nanoparticles from the GI tract after oral administration	Wistar rats	PMMA 130 ± 30 nm	Oral gavage	Found time-dependent deposition and after roughly 15–30 min of exposure nanoparticles were present in the testicles and that they continued to be present for up to 4 days after exposure at the same level.

SNAP, silica Nanoparticle; NP, Nanoparticle; BBB, blood brain barrier; SiNP(s), silica nanoparticles; MNPs@SiO2 (RITC), silica-overcoated magnetic nanoparticles containing rhodamine B isothiocyanate; PMMA, ^{14}C-labelled methyl(2-^{14}C)methacrylate.

organs and tissues. The main objective of the environmental exposure study (Kashiwada 2006) was to evaluate potential toxicological impacts of releasing nanoparticles into the environment.

Types of nanoparticles used

Araujo et al. (1999), Chen et al. (2003), Kim et al. (2006), Kashiwada (2006), and Xue et al. (2005) used a model nanoparticle to assess deposition/translocation and potential toxicity. Three of the four pharmacologic studies used silica coated particles of varying sizes, one pharmacological study used ^{14}C-labelled methyl(2-^{14}C)methacrylate nanoparticles, and the environmental science study used fluorescent latex nanoparticles.

Techniques used

Exposure. Three out of the five studies used mice as the model system, one used Wistar rats, and one study used See-through Medaka (fish). Two out of the three studies using mice used Kunming white mice 17–21 g, while the other study used Male IRC mice (6 weeks old). The three studies using mice administered nanoparticles by injecting them into the abdominal cavity or through the tail. The study using Wistar rats dosed using oral gavage through a plastic stomach tube. The See-through Medaka fish were exposed through nanoparticle-laden water.

The dosing schemes for each of the studies were slightly different. Kim et al. (2006) used three different concentrations of MNPs@SiO_2(RITC), 100, 50, and 25 mg/kg, and administered to the mice intraperitoneally for 4 weeks. Chen et al. (2003) and Xue et al. (2005) fasted the mice for 16 h prior to injecting a suspension with maximum volume of 0.4 ml/20 g bodyweight at different concentrations ranging for 0–225.0 mg/kg bodyweight; two weeks after administration the mice were sacrificed. Araujo et al. (1999) orally administered a volume of 0.5 ml per rat. Kashiwada (2006) exposed three groups of 15 Medaka eggs to 39.4 nm diameter-sized fluorescent particles at 1 mg/l in 10 ml ERM for 3 days with exposures renewed daily.

Findings

Deposition was assessed by weighing the testes. All five studies showed that nanoparticles deposit in the testes and therefore crossed the blood testes barrier, however one study (Kashiwada 2006) found that the amount deposited in the testes was not significant. One study (Araujo et al. 1999) assessed time-dependent deposition and found that after roughly 15–30 min of exposure nanoparticles were present in the testicles and that they continued to be present for up to 4 days after exposure at the same level. None of the studies found toxic effects due to deposition/translocation to the testes.

Toxicity of nanoparticles on male germline cells and/or sperm cells

Objective of studies. *Table II* details the two studies identified pertaining to toxicity of nanoparticles on male germline cells and/or sperm cells. Braydich-Stolle et al. (2005) investigated the suitability of a mouse spermatogonial stem cell line as a model to assess nanotoxicity in the male germline in vitro, while Makhluf et al. (2006) investigated whether magnetite-PVA (ploy-vinyl alcohol) nanoparticles were able to penetrate spontaneously into sperm cells without affecting the sperm motility or its ability to undergo acrosome reaction, two functions which are crucial for successful fertilization.

Types of nanoparticles used

Braydich-Stolle et al. (2005) used the following nanoparticles: Ag (silver) 15 nm, MoO3 (molybdenum) 30 nm, and Al (aluminum) 30 nm. For comparison they assessed the effect of soluble species such as cadmium chloride, silver carbonate, aluminum chloride, and sodium molybdate. Makhluf et al. (2006) used iron oxide nanoparticles coated with PVA.

Techniques used

Braydich-Stolle et al. (2005) assessed cell morphology, mitochondrial function, membrane (LDH) leakage, and apoptosis/necrosis assays using the C18-4 cell line established from type A spermategonia iosolated from 6-day-old mouse testes. Makhluf et al. (2006) dosed bovine sperm and incubated it. Cells were treated with digitonin, which permeabilizes the plasma membrane, or with SDS, which solubilizes the cell's membranes and allows for release of bound and free particles. They looked at acrosome reaction to determine ability to fertilize the egg.

Findings

Braydich-Stolle et al. (2005) found that there was concentration-dependent toxicity for all types of particles tested, whereas the soluble salts had no significant effect; silver nanoparticles were most toxic while Mo03 nanoparticles were least toxic. Their findings suggest that this cell line provides a valuable model with which to assess the cytotoxicity

Table II. Toxicity of nanoparticles on male germline cells and/or sperm.

Author (year)	Objective	Animal/cell line	Nanoparticle, size, concentration	Techniques used	Findings
Braydich-Stolle et al. (2005)	Assess the suitability of a mouse spermatogonial stem cell line as a model to assess nanotoxicity in the male germline *in vitro*.	C18-4 cell line established from type A spermategonia iosolated from 6-day-old mouse testes	Ag (silver) 15 nm, MoO3 (molybdenum) 30 nm, and Al (aluminum) 30 nm. Particles dispersed in phosphate buffered saline at concentrations 5, 10, 25, 50 and 100 ug/ml culture medium. For comparison they assessed the effect of soluble species such as cadmium chloride, silver carbonate, aluminum chloride, and sodium molybdate	Cell morphology, Mitochondrial function, Membrane (LDH) leakage, and Apoptosis/necrosis assays.	Concentration-dependent toxicity for all types of particles testes, whereas the soluble salts had no significant effect. Silver NP were most toxic while Mo03 NP were least toxic. Suggest that this cell line provides a valuable model with which to assess the cytotoxicity of NP in germline cells *in vivo*
Makhluf et al. (2006)	Assess whether magnetite-PVA nanoparticles are able to penetrate spontaneously into sperm cells without affecting the sperm motility or its ability to undergo acrosome reaction, two functions which are crucial for successful fertilization. The importance of showing this is to be able to target these magnetic sperm cells for biomedical and diagnostic applications.	Bovine sperm	Iron oxide nanoparticles coated with PVA (ploy-vinyl alcohol)	Dosed bovine sperm and incubated. Cells were treated with digitonin, which permeabilizes the plasma membrane, or with SDS, which solubilizes the cell's membranes and allows for release of bound and free particles. Used TEM to assess and validate findings. Looked at acrosome reaction to determine ability to fertilize the egg.	Particles bound to the acrosome and a cross-section of the tail revealed high particle binding to the sperm mitochondria. Data indicates that the particles crossed the sperm plasma membrane in order to reach the acrosome and mito-chondria. Particle treated cells are not damaged and that sperm function is normal after treatment. Should therefore be possible to move and target these magnetic sperm cells in the animal body

of nanoparticle in germline cells *in vivo*. Makhluf et al. (2006) found that nanoparticles bound to the acrosome and that a cross-section of the tail revealed high particle binding to the sperm mitochondria. Data indicated that the nanoparticles crossed the sperm plasma membrane in order to reach the acrosome and mitochondria. Nanoparticle-treated cells were not damaged and sperm function, tested using acrosome reactions, was normal after treatment.

Discussion

The seven studies identified provide solid initial data to address the question: Are nanoparticles male reproductive toxicants? The studies reviewed here have shown in mammalian animal models that after injection or ingestion of nanoparticles there is translocation systemically from primary sites of entry across the blood testes barrier, and deposition and bioaccumulation in the testes (Araujo et al. 1999; Chen et al. 2003; Kim et al. 2006; Xue et al. 2005). Furthermore, the presence of small amounts of nanoparticles has also been shown in the glands and interstitial cells of mammalian testes (Chen et al. 2003; Xue et al. 2005). In aquatic species it has been shown, although not statistically significant, that nanoparticles deposit in the testes after gill absorption of contaminated water (Kashiwada 2006). Additionally, there is evidence that germline sperm cells can be damaged due to exposure to metal nanoparticles (Braydich-Stolle et al. 2005). While there is great distinction between the types of studies that were found (i.e., pharmacological or environmental) in this review, one main similarity is that none of the papers identified used human cells or tissues in their exposure models.

In addition to the findings from the research reviewed here, the chemical properties of many nanoparticles are similar to some persistent environmental contaminants that are known endocrine disruptors such as organochlorines. For example, the lipophilic and hydrophobic properties of fullerenes (Thomas & Sayre 2005) have been identified and results reported by Oberdörster (2004) suggest that exposure to C_{60} fullerenes can potentially cause cellular damage, specifically lipid peroxidation in the brains of largemouth bass.

In order to maximize future research efforts, it is important that the field advances in a more planned and deliberate way with respect to model species, methods of exposure, dosing practices, size of nanoparticles, and target organs assessed. Toxicological studies are needed to understand the absorption, distribution, metabolism, and excretion of the various nanoparticles being used today, as well as the coatings being placed on these nanoparticles. Studies are also needed to determine the exact physiochemical characteristics of these nanoparticles including their potential impact on the hypothalamus, gonadal, and pituitary axis to help determine their potential as reproductive toxicants, based on our current knowledge of the chemical properties. In order to perform human studies it will be critical to identify exposure biomarkers for the biomonitoring of nanoparticle compounds in blood, urine, semen, etc.

Conclusion

This literature review on the current state of knowledge about the deposition/translocation of nanoparticles to the testes and male germline cells, as well as the potential cytotoxic effects of nanoparticles on male germline cells and/or sperm identified seven studies that provide evidence to support the notion that nanoparticles deposit in the testes and they have adverse effects on male germline cells. Further investigations need to advance in a concerted way to definitively substantiate or refute potential male reproductive harm due to nanoparticles.

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