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RESEARCH ARTICLE

The Reproductive Toxicity of Silver Nanoparticles in Testis

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Abstract

The reproductive toxicity of silver nano-particles (AgNPs) is the hotspot in recent years, but the mechanism is not entirely clear.There are some papers and experiments confirmed that AgNPs can be transported through the blood-testicular barrier(BTB),accumulated in testis, caused male reproductive toxicity. In the review, we summarized that significant nanotoxicity mechanisms of AgNPsand some other possible mechanisms in male reproductive system, such as oxidative stress, apoptosis and hormone regulation. Meanwhile, we conjectured several mechanisms of AgNPs which needs further research to be confirmed, such as pyroptosis,endoplasmic reticulum stress(ERs),mitophagy and epigenetics. The review sums up the toxicity and mechanisms of AgNPs in seven aspects.

Keywords: Silver Nanoparticles; Testis; Oxidative Damage; Pyroptosis; ERS; Mitochondrial Autophagy

Introduction

AgNPs is a novel material based on nanotechnology. Because of its unique characteristics, including large specific surface area, small dimension, strong surfactancy, excellent catalytic performance, AgNP is wide-spread used in medicine, diet, ceramics, optics, textile, cosmetics, catalyst, semiconductor, purifying water quality, and etc [1]. AgNP is also added in fodder instead of antibiotic due to bacterial resistance [2] .Nowadays, more and more products containing AgNPs appear in daily life, which makes people easier to be exposed to AgNPs through respiratory tract, alimentary canal and skin. Most researchers are focused on the toxicity of AgNPs related to liver, kidney, spleen, lung and brain.However,there are still a few reports show that AgNPs have negative effects on testis and related factors, and hence damage reproductive system [3-5] Because the exact mechanism of AgNP in testis was poorly understood, we write this review to summarize the mechanism of AgNP's nanotoxicity in testis to provide more inspiration and alarms in AgNP's application.

The distribution and transferring of AgNPs

Many literatures and studies have shown that AgNPs can enter tissues of the body through various routes, such as the gastrointestinal tract, respiratory tract, and injection. Compared with injection, the accumulated AgNPs by the oral route seem to be relatively low [6]. Not only can it accumulate in the liver, spleen, kidneys, brain and lungs, but it can also cross the blood-testis barrier and remain in the testis. Small-sized AgNPs are more active to exert biological or toxicological responses [7], and are more likely to accumulate in various organs through blood. Park, *et al.* found that after the application of 22nm and 42nm AgNP, Ag accumulated in all tissues, and testicular Ag was comparable or higher than other tissues. No Ag was detected in the testes after 71 nm AgNP administration. After administration of 323 nm AgNP, no Ag was detected in any tissue. This shows that the size of AgNPs has an effect on organ accumulation [6]. Scholars Asare *et al.* followed and observed that rats were orally administered AgNPs and found that the concentration of AgNPs in the testes increased in dose-dependent after 28 days [8]. After 28 days of oral administration of AgNPs in male rats, regardless of the size of AgNPs, the level of Ag tissue measured was the highest after 28 days, and the Ag level gradually decreased after 4 months of recovery. After stopping the administration, the levels of Ag in blood, liver, kidney and spleen decreased, but the levels of Ag in testes and brain did not decrease. This shows that AgNPs continue to accumulate in the testes and brain and cannot be ruled out or dissipated [9]. When bucks were injected with AgNPs (45nm) intravenously, ejaculated spermatozoa were taken

for detection. TEM analysis showed that NPs existed in the cytoplasmic residues, nuclear segments, acrosomes and axons of sperm samples. AgNPs can cause sperm ultrastructural damage and affect the integrity of sperm acrosomes and mitochondria [10].

The mechanism of oxidative damage induced by nanosilver in testicular cells

According to the literature, classical oxidative stress involves signaling pathways such as Keap1-Nrf2-ARE, STAT3/NF-κB, and SIRT1-FOXO. In the mechanism of oxidative damage induced by AgNPs in testicular cells, there are few oxidative stress pathways. In the study, the above classical signaling pathway of oxidative stress may be involved in the cytotoxic mechanism induced by AgNPs, which is worthy of discussion in the future.

The production of reactive oxygen species (ROS) and activation of oxidative stress are usually regarded as the mechanism about reproductive toxicity which induced by AgNP. There are some many studies have shown that AgNPs can increase ROS in the testis, trigger oxidative stress and cause damage to the testis [3-5]. Habas, *et al.* found that AgNPs can enter the sertoli cells, increase the expression of p53 mRNA in the sertoli cells and decrease the expression of SOD, CAT, GPX and bcl-2 mRNA in the cells, resulting in accumulation of intracellular ROS and damage to supporting cells. Asare' expriments [12] showed that the expression of antioxidant genes SOD1, SOD2 and SOD3 in testis increased in mice after infecting with AgNPs (20, 200nm) at 5mg/kg, indicating that AgNPs induced oxidative stress in mouse testis. Almansour, *et al.* [13] proposed that for different particle size of AgNPs, the nano-toxicity is also different.AgNPs can produce ROS in the testis.The smaller the particle size, the more ROS are produced. Ong, *et al.* [14] applied AgNPs (20nm) to Drosophila at agradient concentration of 0-5mg/L, which resulted in a decrease of germline stem cells and an increase of ROS production in the testis. Coccini, *et al.* [15] also found that dripping AgNPs into the lungs of rats caused testicular damage and significantly increased the expression of antioxidant genes such as SOD, Gpx1, FMO2 and GAPDH in the testis. It can be seen that the mechanism of oxidative damage caused by AgNPs to reproductive toxicity may caused by a certain dose of AgNPs.The exposure of AgNPs can produce ROS production in testicular cells and up-regulate the expression of antioxidant genes, finally resulting in an imbalance between oxidation and antioxidation of testicular cells, leading to oxidative stress. The oxidative stress eventually causes damage to the reproductive system.

Nano-silver induced cell pyroptosis mechanism

Pyroptosis is a recently discovered pattern of programmed cell death that is dependent on the inflammasomes, also known as inflammatory necrosis. When the cells are infected or pathologically damaged, the NLRP3 inflammasome assembly is induced to activate caspase-1, which promotes the maturation and release of IL-1β and IL-18, and finally initiates the inflammatory cascade, leading to cell death. This is a classical pathway for cell death[16].Therefore, targeting NLRP3 inflammasome has become a hot spot for the development of therapeutic drugs for related diseases [17].

Unfavorable factors in the inherited and environmental conditions can induce cell death, including not only the pathogen-associated molecular pattern (PAMP) of the pathogenic microorganism, but also the damage-related molecular pattern (DAMP) from the body itself [18].Studies have found that Ag NPs can induce cell inflammatory death to mediate a variety of biological toxic effects [19].For example, After exposure to silver nanoparticles, production of IL-1b, a critical cytokine involved in induction of innate immunity, significantly increased as particle size decreased, finally induced cytotoxicity [20].

It can be seen that AgNPs can cause many types of cell death depended by inflammasomes, but whether AgNPs can cause germ cell pyroptosis has not been reported so far, and this field will be a hot spot for future research.

Nano-silver induced cell ERS mechanism

In mammalian cells, endoplasmic reticulum stress (ERS) signals are usually sensed by three major endoplasmic reticulum resident transmembrane molecules, IRE1, PERK and ATF6 [21].These three stress sensors are normally inhibited in non-stressed cells by binding to immunoglobulin via endoplasmic reticulum mate protein GRP78 (78 KDa glucose-regulating protein, also known as BiP), which can be activated by GRP78 dissociating and binding to unfolded proteins in the endoplasmic reticulum, causing an unfolded protein reaction that causes ERS [22] .

With cadmium induced mice testis ERS, the endoplasmic reticulum in testicular germ cell molecular chaperoe GRP78 and ATF6 expression quantity were rised significantly. The PERK pathways downstream molecules --- phosphorylated eIF2α and CHOPalso raised obviously, the expression of promoting apoptosis gene Bim, BAX and Bad quantity increased significantly.With PBA pretreatment testicular germ cells, it can suppress germ cell apoptosis.The results indicate that ERS mediates cadmium-induced germ cell apoptosis [23].There is a study inducing ERS in testicular Leydig cells by continuous heat stress, and the expression of ERS critical genes GRP78/BiP and CHOP are up-regulated. However, the sterol synthase 3 beta-hydroxysteroid dehydrogenase (3β-hsd) in Leydig cells decreased with the increase of temperature, resulting in decreased testosterone synthesis and abnormal sperm-genesis [24]. These results suggest that ERS is closely related to the survival of testicular cells.

ERS was found to be activated after exposure to nanoparticles, especially metallic nanoparticles [25]. Huo, *et al.* [26] showed cell-type dependent activation of ERS pathway when exposed to AgNPs, and normal lung cells (16HBE cells) were more likely to induce ERS activation response than human umbilical vein endothelial cells (HUVECs) and HepG2 cells (human liver cancer

cells).Yang, *et al.* [27] demonstrated that oral administration of Zno nanoparticles could induce endoplasmic reticulum swelling and ribosomal degranulation in mouse liver, and observed hepatocyte necrosis, suggesting that ERS may be the mechanism of liver injury induced by nanoparticles in vivo.Similarly, Yu, *et al.* [28] also found a dose-dependent increase in endoplasmic reticulum swelling and endoplasmic reticulum stress markers in mice inhaled Tio2 nanoparticles. These evidences show that AgNPs and other nanomaterials can induce endoplasmic reticulum stress in a variety of cells, but whether AgNPs can induce endoplasmic reticulum stress in testicular cells has not been reported so far, and this field will be the focus of future research.

Nano - silver induced mitochondrial autophagy mechanism

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Mohan, *et al.* [29-31] proposed that sperm itself contains proteins required by autophagy and mitochondrial autophagy. First, it is used to eliminate mitochondria from sperm in zygote. Second, it is used to ensure exclusive inheritance of mitochondrial DNA from mother side. Thus, it can be seen that mitochondrial autophagy plays an important role in reproductive inheritance.

Mitophagy is the process of cells removing damaged or aging mitochondria and recycling their components. PINK1 / Parkin is a classic signaling pathway that activates mitochondrial autophagy. After phosphorylation, Parkin translocates cytoplasm to mitochondria, thereby ubiquitinating a series of mitochondrial outer membrane proteins and eventually inducing mitochondrial autophagy. Studies have shown that [30] nanometer zinc oxide particles (ZnO NP) can increase the content of PINK1 and induce Parkin to transfer from cytoplasm to mitochondria, thereby damaging the mitochondria of mouse small glioma cells (bv-2 cells) and triggering mitochondrial autophagy. Wang, *et al.* [31] found that while ZnO NP reduced the mitochondrial membrane potential in CAL 27 cells, it also activated the PINK1 / Parkin signaling pathway in the cells, leading to mitochondrial autophagy in CAL 27 cells.In addition, Lee, *et al.* [29] showed that when embryonic fibroblasts (NIH3T3 cells) were infected with AgNPs, LC3 and p62 protein levels in NIH3T3 cells could be induced to increase, indicating the production of autophagosomes induced by AgNPs. These results indicate that AgNPs and other nanomaterials can induce mitochondrial autophagy in a variety of cells, but whether AgNPs can induce mitochondrial autophagy in testicular cells has not been reported so far, and this field will be a question to be studied in the future.

Epigenetic toxicity induced by silver nanoparticles

Epigenetic studies include DNA methylation, histone modification, and noncoding RNA regulation. Abnormalities in any one part will affect gene expression [32].Studies have shown that AgNPs poisoning can cause epigenetic changes in many types of cells. For example, DNA methyltransferase is a family of enzymes that catalyze DNA methylation. Nanoparticles can change the pattern of DNA methylation by regulating the expression and activity of DNA methyltransferase (DNMTs) [33].Nanoparticles also block gene expression by inducing hypermethylation of the promoter, which promotes gene expression [34]. MYtych, *et al.* [35] demonstrated for the first time that AgNPs could induce increased levels of 5-methylcytosine (5-mc) and DNA methyltransferase 1, 3a and 3b (DNMT1,DNMT3a and DNMT3b), resulting in significant DNA methylation in mouse hippocampal neuron cell lines (HT22 cells).Gliga, *et al.* [36] exposed human lung cells to AgNP (10nm, 1 mug /ml) for 6 weeks, and transcriptome analysis revealed marginal effects of DNA methylation. Blanco *et al.* [37]found that AgNPs (100, 200 mug /ml) increased the overall DNA methylation level and induced histone H3 deacetylation after 24 and 72 hours of exposure to human lung cancer cells.Eom, *et al.* [38] showed that AgNPs could induce the up-regulation or down-regulation of miRNAs expression in Jurkat T cells, thereby causing toxic effects in Jurkat T cells.Huang, *et al.* [39] applied AgNPs to human skin fibroblasts (HDF) after they were infected, and down-regulated the expressions of SOS1 and CDC25B through mir-424-5p in the MAPK signaling pathway, which blocked the cell cycle and promoted apoptosis.

The above studies have shown that AgNPs can induce epigenetic changes in a variety of cells, but whether AgNPs can induce epigenetic changes in testicular cells has not been reported in the literature. Therefore, whether AgNPs can induce epigenetic toxicity in testicular cells, and what is the specific mechanism? This field may become a hot spot of future research, which needs further exploration by scholars.

Nanometer silver regulates reproductive hormone levels

AgNPs induced changes in reproductive system hormone levels is one of the mechanisms that impair reproductive function. Han et al[4] showed that the expression levels of steroid-generated 3/3-hsd, 17/3-hsd, Cyp17al and Cyp19al genes in leydig cell in ICR mice infected with AgNPs were significantly reduced, indicating that AgNPs could affect the function of leydig cell by inhibiting

the synthesis of male steroid hormones. Baki's experiment confirmed[40] that after feeding Wistar rats AgNPs (60nm, 25, 50, 100 and 200 mg/kg/d) for 45 days, the serum testosterone level of the rats decreased, while the luteinizing hormone (LH) level increased, and the number of leydig cell decreased, sperm motility decreased and sperm morphology changed.Ahmed. *et al.* [41] found that exposure to AgNPs (< 100nm, 100, 1000mg/kg/d) reduced serum testosterone levels in Wistar rats, which was caused by AgNPs induced leydig cell damage.In the study of Dziendzikowska, *et al.* [42], intravenous injection of Wistar rats with AgNPs (20nm, 5mg/kg, 10mg/kg; and 200nm, 5mg/kg) significantly reduced the levels of testosterone and DHT in plasma and testes, and significantly down-regulated the mRNA expressions of corticosteroid-related genes Star, Cyp11al, Hsd3bl, Hsd17b3 and Srd5al. The research results show that a certain dose of AgNPs canister can cause changes in the level of reproductive system of male hormones, mainly by inhibiting the steroids to generate the expression of genes involved in metabolism, make the synthesis of steroid hormones affected, which affect the function of leydig cells secrete testosterone, resulting in a decline in sperm motility and sperm morphological change.

Discussion

In this paper, we summarized the toxicity mechanism of AgNPs on male reproductive system . The results of different doses and different ways of infection showed that AgNPs could penetrate the blood-testosterone barrier into the testis and accumulate in the testis, which had adverse effects on germ cells.The most important mechanism of toxicity is through oxidative stress, apoptosis and the regulation of hormone levels in the reproductive system.As for the mechanism of AgNPs causing pyroptosis, endoplasmic reticulum stress, mitochondrial autophagy and epigenetic toxicity, there have been a few reports in other systematic experiments. About the toxic mechanism of the male reproductive system, further studies on AgNPs are needed to fill in relevant data and evidences to clarify the toxic mechanism of AgNPs.

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