Fish and Rat as Models to Assess Environmental Toxicity of Silver Nanoparticles

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Abstract
Nanoparticle-sized particles of less than 100 nm in diameter are attracting the scientific community due to their wide range of new applications in various fields including biophysics, material and medical sciences. Nanoparticles of noble metals such as silver have been found to reveal significantly distinct physical, chemical and biological properties from their bulk counterparts. As industrial production of nanoparticle-sized particles is increasing day by day, their dissemination in natural environments is also at an increase. This is leading to substantial water and soil pollution. Finding the adverse effects of such nanoparticle-sized particles on different faunal species is thus of utmost importance. In the present paper, toxic effects of silver nanoparticles on fish and rat models have been presented so as to have a view about the type of the damage posed by them. This study will not only add to the existing knowledge about the toxic effects of silver nanoparticles on faunal species but also help in formulating future pollution controlling programs.

Keywords: Silver Nanoparticles, Toxicity, Environmental Pollution, Environmental Exposure, Genotoxicity, Oxidative Stress, Fish, Rat

1. Introduction

In recent years, various novel nanomaterials have received much attention due to their great potential for applications in agriculture, food safety, and food packaging [1-7]. Relatively little is known about the behavior and toxicity of nanoparticles in the environment. Nano-sized particles are near atomic scale size and have at least one dimension in the range of 1-100 nm. Nano-sized particles are attracting the scientific community for their wide range of applications in various fields [5-7]. Nanoparticles can exhibit properties that differ markedly from those of bulk materials, as a result of small particles dimension, high surface area and quantum confinements. Silver nanoparticles (SNPs; Figure 1) are likely to enter the aquatic ecosystems because of their wide industrial production and multiple applications. This can lead to serious health issues for humans and aquatic species [8]. During recycling or disposal, these engineered nanoparticles are also likely to expose to the environment. Although SNPs are increasingly used in various consumer products and produced in industrial scale [9], information on harmful effects of nanosilver to environmentally relevant organisms is still scarce. Once released, these engineered nanoparticles undergo transformations via biotic and abiotic processes and are likely to become yet another anthropogenic source. Toxicological aspects of silver nanoparticles are yet to be found particularly in humans. Here in this paper, a review of toxicological aspects of SNPs has been presented particularly in fish and rat models.
Figure 1. 40nm silver nanoparticles [10]

2. Toxicological Studies among Fishes

Fish is an important research animal model to find out the toxicity of various water dissolved chemicals and particles as they may present the direct exposure results. A number of researches have been found using fish as model for finding toxicity of SNPs [11-15]. Zebrafish has been used as a model to find various recent findings (Figure 2; Reprinted from Marelli and Persani [16]) SNPs have been found lethal to Zebrafish (Danio rerio) [11]. Asharani et al. [17] studied the toxicity of silver nanoparticles in Zebrafish embryos. The results suggested that SNPs induce a dose dependent toxicity in embryos which in turn hinders normal development in the fish. Bar-Ilan et al. [14] assessed the toxicity of multi-sized gold and silver nanoparticles in Zabrafish embryos. Using colloidal silver (cAg) and gold nanoparticles (cAu) in a panoply of sizes (3, 10, 50, and 100 nm) and a semi-quantitative scoring system, it was found that cAg produces almost 100% mortality at 120 h post-fertilization, while cAu produces less than 3% mortality at the same time point. They concluded that Zebrafish embryo model should lead to the identification of nanomaterial characteristics that afford minimal or no toxicity and guide more rational designs of materials on the nanoscale. Similarly, Bilberg et al. [11] studied in-vivo toxicity of silver nanoparticles and silver ions in Zebrafish. Acute toxicity of nanosilver to Zebrafish was studied and compared with the toxicity of nano silver and silver ion. To investigate exposure related stress, the fish behaviour was observed for both nanosilver and ionic silver treatment. The observations revealed increased rate of opercular movement and surface respiration after nanosilver exposure, suggesting respiratory toxicity.

Figure 2. A schematic illustration showing recent finding while using zebrafish as a model (Reprinted from Marelli and Persani [16])
Lee et al. [18] synthesized and characterized purified and stable (non-aggregation) silver nanoparticles (SNPs, 41.6 ± 9.1 nm in average diameter) and utilized early developing cleavage-stages of Zebrafish embryos as in-vivo models to probe the diffusion and toxicity of SNPs. At lower concentrations of the NPs (<0.02 nM), 75-91% of embryos died. At higher concentrations of NPs (≥0.20 nM), 100% of embryos died. At the concentrations in between (0.02-0.2 nM), embryos developed into various deformed adults. Lee et al. [19] functionalized the surfaces of SNPs (SNPs, 11.7 ± 2.7 nm in diameter) with three biocompatible peptides (CALNNK, CALNNS, CALNNE) to prepare positively [Ag-CALNNK NPs (+zeta)], negatively [Ag-CALNNS NPs (-2zeta)], and more negatively charged NPs [Ag-CALNNE NPs (-4zeta)] respectively. The study found that all three Ag-peptide NPs passively diffused into the embryos via their chorionic pore canals, and stayed inside the embryos throughout their entire development (120 h). NPs showed charge-dependent toxic effects on embryonic development, showing that the positively charged Ag-CALNNK NPs (+zeta) were the most biocompatible while the negatively charged Ag-CALNNE NPs (-4zeta) were the most toxic.

Muth-Kohne et al. [20] treated Zebrafish embryos with SNPs (particle size: >90 % <20 nm) and AgNO3 in ISO water for 48 h and consequently displayed effects such as delayed development, tail malformations and edema. A TEM analysis confirmed uptake of the SNPs, and the distribution within the embryo suggested absorption across the skin. An increased fish embryo toxicity due to sewage treatment plant (STP) effluents with increasing SNPs influent concentrations identified the accumulation of SNPs in the STP as a potential source of effluent toxicity. Van et al. [21] studied the molecular mechanism of toxicity of SNPs in Zebrafish embryos. The result supported the hypothesis that the toxicity caused by SNPs is principally associated with bio-available silver ions in exposed Zebrafish embryos. Bowman et al. [13] studied the effects of SNPs in Zebrafish and E. coli to compare the toxicity based on total surface area versus mass concentration of particles. Several diameters of SNPs (20, 50, 110 nm) as well as AgNO3 were chosen as experimental treatments. Treated Zebrafish embryos exhibited anomalies of the heart including slower heart rates and pericardial edema.

Lee et al. [19] studied the toxicity of citrate capped SNPs in Cyprinus carpio (common carp). The result showed that SNPs agglomerated in fresh water with size remaining greater than 100 nm. SNPs had no lethal effect on fish after 04 days of exposure. Biochemical analysis showed that enzymatic activities in the brain of fish exposed to 200 µg L⁻¹ of SNPs were significantly reduced. Varied antioxidant enzyme activity was recorded in the liver and gills. Kim et al. [22] studied the developmental toxicity of silver nanoparticles and release of silver ions in the presence of humic acid on Japanese medaka embryos. In this study the effect of two differently prepared SNPs (freshly prepared and aged) on Japanese medaka embryos. The results suggested that aged SNPs release more silver ions and are more toxic than fresh SNPs and humic acid plays a role in reducing the toxicity of aged SNPs. Similarly, Kwok et al. [23] studied the uptake of SNPs and toxicity to early life stages of Japanese medaka. The results revealed that SNP is a source of toxic Ag ions and gills are the principal organs for uptake of silver ions. Cho et al. [24] studied the step-wise embryonic toxicity of SNPs on Oryzias latipes. The results showed that SNP exposure caused severe developmental toxicity in medaka embryos and the toxicity levels were enhanced at certain developmental stages which should be taken into consideration in assessment of metallic NP toxicity to embryos.

3. Toxicological Studies using Rats as Models

Rats have been used as model animals in different studies based upon the assessment of toxic profiles of a variety of compounds [25,26]. Rats have been taken as nearest animal models to humans which have like physiological effects. A number of studies were found which used rats as animal models to assess toxicity of SNPs.

De Jong et al. [25] determined the potential systemic toxicity of SNPs with different sizes (20 nm and 100 nm) and a 28-days repeated dose toxicity study was performed in rats using intravenous administration. Treatment with a maximum dose of 6 mg/kg body weight was well tolerated by the animals. However, both for 20 nm and 100 nm SNP growth retardation was observed during the treatment. At histopathological evaluation brown and black pigment indicating SNP accumulation was noted in spleen, liver, and lymph nodes. Clinical chemistry indicated liver damage with increased alkaline phosphatase, alanine transaminase, and aspartate transaminase. Ji et al. [26] studied the inhalation toxicity of SNP in Sprague-dawley rats. In this inhalation test, rats were studied over a period of 28 days. The animals were exposed to SNPs for 6 hr/day, 5days/week for 4 week. Rats did not show any significant changes in body weight, haematology or blood biochemical
values. This study concluded that exposure to SNPs at a concentration near to 100 µg/m³ (which is ACGIH silver dust limit) did not appear to have any significant health effect. Kim et al. [27] studied the oral toxicity, genotoxicity and gender related tissue distribution of SNPs in Sprague-dawley rats. Oral toxicity of SNPs over 28 days in rats was studied. SNPs did not induce any genetic toxicity in male or female rat bone marrow under in-vivo conditions. The tissue distribution of SNPs did show a dose-dependent accumulation of silver in all tissues. A gender related difference in the accumulation of SNPs was noted in the kidney with a 2 fold increased accumulation in the female kidneys as compared to the male kidneys.

Sung et al. [28] studied the sub-chronic inhalation toxicity of SNPs. Study revealed that target organs for SNPs were the lung and liver in the male and female rats. The animals were exposed to silver nanoparticles (average diameter 18-19 nm) for 6 h/day, 5 days/week, for 13 weeks in a whole-body inhalation chamber. Bile-duct hyperplasia in the liver increased dose dependently in both the male and female rats. Histopathological examinations indicated dose-dependent increase in lesions related to silver nanoparticle exposure, including mixed inflammatory cell infiltrate, chronic alveolar inflammation, and small granulomatous lesions. Kim et al. [29] tested the oral toxicity of silver nanoparticles (56 nm) over a period of 13 weeks (90 days) in F344 rats and the results showed a significant decrease (P < 0.05) in the body weight of male rats after 4 weeks of exposure. Significant dose-dependent changes were found in alkaline phosphatase and cholesterol for the male and female rats, indicating that exposure to more than 125 mg/kg of silver nanoparticles may result in slight liver damage. Histopathologic examination also revealed a higher incidence of bile-duct hyperplasia, with or without necrosis, fibrosis, and/or pigmentation, in treated animals. Sung et al. [30] assessed the toxicity of SNPs and their effect on rat liver mitochondrial bioenergetics. The results revealed that SNPs cause impairment of mitochondrial functions, mainly due to alteration of mitochondrial membrane permeability. This results in an uncoupling effect on the oxidative phosphorylation system. Thus, mitochondrial toxicity may have a central role in the toxicity resulting from exposure to SNPs. Maneewattanapinyo et al. [31] evaluated acute toxicity of colloidal SNPs and reported that colloidal SNPs could be relatively safe when administered orally, to eye and skin of animal models including rat and guinea pig for short period of time. Van der zande et al. [21] studied the distribution, elimination and toxicity of SNPs and silver ions in rats after 28 days of oral exposure. Rats were exposed to <20 nm non-coated, or <15 nm PVP-coated silver nanoparticles ([Ag] = 90 mg/kg body weight (bw)), or AgNO₃ ([Ag] = 9 mg/kg bw), or carrier solution only. Result showed that oral exposure to SNPs appears to be very similar to exposure to silver salts. Biochemical markers and antibody levels in blood, lymphocyte proliferation and cytokine release, and NK-cell activity did not reveal hepatotoxicity or immunotoxicity of the silver exposure.

Hong et al. [32] studied the combined repeated-dose toxicity of SNPs with reproduction /developmental toxicity screening test in rats. In this test, the rats were treated with 62.5, 125 and 250mg/kg of citrate capped SNPs once a day for 42 days in male and 52 days in female. No death was observed in any group but alopecia, salivation, and yellow discoloration of the lung was observed in few rats. Toxicity endpoints of reproduction/development screening test including mating, fertility implantation, delivery and foetus were measured and no evidence of toxicity was found. Kim et al. in [33] evaluated the genotoxicity, acute oral and dermal toxicity, eye irritation, dermal irritation and corrosion and skin sensitization of commercially manufactured SNPs. In acute oral and dermal toxicity tests using rats, none of the rats showed any abnormal signs or mortality at a dose level of approximately 2000 mg/kg. The SNPs were not found to induce genotoxicity in a bacterial reverse mutation test and chromosomal aberration test, although some cytotoxicity was observed.

4. Conclusions

After going through the above reported studies, it can be concluded that fish and rat models can be effectively used in assessing the toxicity of various kinds of nanoparticles found in natural water bodies. Fish and rat models can be used to assess different sensitive parameters followed by nanoparticle exposure which include blood parameters, histochemistry, bio-chemical analysis, toxicological analysis and behavioral patterns. Both fish and rat can be used as sensitive models for assessing the toxicity of silver nanoparticles.

Conflict of interest

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