Use of Silver Nanoparticles: Opportunities & Challenges

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Abstract

This review article evaluates and summarises the present knowledge on the behavior and the biological effects of Silver Nanoparticles (Ag NPs) to organisms. Though, Silver Nanoparticles have distinctive physico-chemical properties, including a high electrical and thermal conductivity, *In vitro* studies demonstrated that Ag-NP are cytotoxic by their effect on cellular metabolism and membrane integrity, and inhibit embryonic stem cell differentiation.

Introduction

Nanotechnology has been defined as using materials and structures with nanoscale dimensions, usually in the range of 1–100 nm (Zhang, 2003). As interest in the potential benefits of nanomaterials have increased, the toxic effects resulting from use or unintentional release into the environment are concerned (Moore, 2006). Silver Nanoparticles have distinctive physico-chemical properties, including a high electrical and thermal conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity and non-linear optical behaviour (Capek, 2004; Frattini et al. 2005). These properties make them of potential value in inks (Perelaer et al. 2009; Tay and Edirisinghe, 2002), microelectronics (Wu et al. 2006), and medical imaging (Jain et al., 2008). However, it is the exceptional broad spectrum bacteriocidal activity of silver (Luoma, 2008; Ratte, 1999; Silver, 2003; Silver et al., 2005) and relatively low cost of manufacturing of Ag NPs (Capek, 2004), that has made them extremely popular in a diverse range of consumer materials, including plastics, soaps, pastes, metals and textiles (Frattini et al., 2005) increasing their market value.

Despite the rapidly growing presence of silver-containing nanoproducts on the market (Wijnhoven et al. 2009; Woodrow Wilson International Centre for Scholars 2011; Lem et al 2012; Dekkers et al. 2007), there is only limited information on the possible risks of exposure to silver nanoparticles. *In vitro* studies demonstrated that Ag-NP are cytotoxic by their effect on cellular metabolism and membrane integrity, and inhibit embryonic stem cell differentiation (Park et al. 2011).

Systemic and immunotoxicity of silver nanoparticles in an intravenous 28 days repeated dose toxicity study in rats

To avoid limited systemic exposure due to the cellular barriers present in lung and Gastrointestinal (GI)-tract, intravenous administration of nanosilver was used, to evaluate its potential systemic toxicity. The *in vivo* toxicity of two sizes of Ag-NP (20 nm and 100 nm) was investigated in a repeated dose toxicity study after intravenous administration for 28 days. Special emphasis was on the effects of the spleen as part of the immune system as previous results showed accumulation of Ag-NP in the spleen (Lankveld et al. 2010).

The following organs were examined and sampled: adrenals, brain, bone marrow, small intestines (duodenum, jejunum, ileum), large intestines (caecum, colon, rectum), heart, kidney, liver, lung, lymph nodes (mesenteric and popliteal), esophagus, pituitary, spleen, stomach, testis (or ovaria), and thymus.

Macroscopic evaluation and effect on organ weights

Macroscopic examination of the rats revealed enlarged brownish colored spleens and livers, and enlarged and dark colored lymph nodes in the highest dose groups.

Heart, kidneys, adrenals, brain, testes and epididymis were weighed and showed no differences in the different dose groups (Ag-NP 20 nm) compared to the control, whereas for liver a weight increase and for the thymus a weight decrease was observed. After treatment for 28 days with 100 nm Ag-NP (6 mg/kg b.w.) in both male and female animals spleen weight was similarly increased compared to control (phosphate treated) animals BMD analysis showed for the liver an increase in weight for males and females after treatment with 20 nm Ag-NP.

Histopathology

In the histopathological evaluation pigment was observed in various organs including spleen, liver, and lymph nodes. The pigment itself was not further identified but could be ascribed to the presence of silver nanoparticles in the various organs. After administration of 20 nm Ag-NP the pigment in the spleen was mainly observed in the red pulp, while after 100 nm Ag-NP the pigment was observed in both the red and white pulp of the spleen. In the liver the pigment (nanoparticles) was present in the Kupffer cells lining the walls of the venous sinusoids. In the lung, small granulomas were observed partly caused by the injection technique as indicated by the presence of hair fragments. Some granulomas contained black pigment after injection of 100 nm Ag-NP (Wim et al. 2013).

Evaluation of the toxic impact of silver nanoparticles on *Japanese* medaka (*Oryzias latipes*)

To determine the differences in the toxic responses to Ag-NPs and Ag^+ ions, $AgNO_3$ was dissolved in Milli-Q water and used at an equivalent elemental Ag mass. Prior to use, the size, zeta potential and surface areas of the Ag-NPs were characterized. The distribution, size and shape of the particles were obtained by transmission electron microscopy (TEM) and X-ray diffraction (XRD) analysis. The zeta potential of the suspended particle solution was measured using an electrophoretic light scattering spectrophotometer.

Initially, acute toxicity tests were carried out to determine the dose dependent response curves for lethality when Medaka are exposed to Ag-NPs or $AgNO_3$. Flow-through toxicity tests were conducted with different concentrations of Ag-NPs and $AgNO_3$ up to *c.a.* 50μg/l equivalent elemental Ag throughout a 96 h exposure to determine the dose dependent response curves for lethality with *Oryzias latipes*. The percent mortality for the fish increased with longer exposure times and in proportion with higher dosages of either toxicant. In most cases, however, Ag-NPs caused a more

severe toxic effect than $AgNO_3$. The results of lethal toxicity at 96 h exposure suggest that Ag-NPs and $AgNO_3$ are only slightly different in their lethality. However, significant differences in the percent survival during exposure to Ag-NPs and $AgNO_3$ were seen for the earlier exposure time points (*e.g*., 24, 48, 72 h).

To study changes in the gene expression levels, two concentrations of Ag-NPs, 1μg/L (low dosage) and 25μg/L (high dosage), were selected for the tests. The higher dosage, 25μg/L,was chosen based upon the findings in the acute toxicity tests, which showed this to be the lowest concentration causing loss in viability (LOEC) while the lower concentration, 1μg/L, was selected to evaluate the environmental toxicity of Ag-NPs at a low level exposure. Additionally, 1.58 and 39.46μg/L Ag-NO_3 were also tested. The concentrations were selected to offer an equivalent mass of Ag as in the low and high Ag-NP exposures, respectively. In fact, the concentration leading to 50% loss in viability (LC50) with the Ag-NPs and $AgNO_3$ were 34.6±0.9 and 36.5±1.8μg/l elemental silver, respectively, when a 96 h exposure was used.

In this study, the 18S rRNA was chosen as the endogenous control gene. The expression level of the 18S rRNA was constant, with an expression ratio of nearly one for all the samples. Therefore, using this gene as a reference, changes in the expression levels of six biomarker genes were evaluated and compared after the Medaka were exposed to the Ag-NPs and $AgNO_3$.

Metallothionein is a biomarker often used as an indicator of metal exposure since it is highly specific and sensitive to metals and is induced in response to elevated metal concentrations in tissues or living cells (Olafson *et al*, 1988). In this study, a statistically significant induction of MT expression (6.0-fold increase) was observed 1 day after the fish were exposed to 25μg/L Ag-NPs, which was much higher than the response seen with an equivalent mass of metallic silver from $AgNO_3$ (2.2-fold). Additionally, all test concentrations for both chemicals, except for 1μg/L Ag-NPs, led to increased mRNA levels of this gene (at least 2.2-fold when compared to control) during the first 2 days of exposure. Based upon these results, induction of the MT mRNA levels in the liver is abrupt but not pro-longed, showing an initial increase during the first couple of days and then a decrease back to a basal level expression with longer exposure times (>4 days). A comparison between the MT expression levels when exposed to equivalent elemental silver amounts of Ag-NPs and $AgNO_3$ show that there is a significant difference between the toxicants during short exposure times but this is lost as the exposure time is extended. These results are in contrast to those of the heat shock protein (HSP70), for which only the high dosage of Ag-NPs was found to activate the gene and increase the mRNA levels about 2.5-fold during the first day of exposure. However, a depressed mRNA level (about 3.5-fold) was seen with the 4-day exposure. In contrast, the addition of $AgNO_3$ led to a repressed expression of this gene at all tested dosages up to a 2-day exposure when compared to the control. Longer exposures to both chemicals did not result in any significant induction of mRNA levels when compared with those levels of the controls.

Transferrin (TF), an immune system-related gene, was down-regulated in the fish that were exposed to Ag-NPs. The results with 25μg/L showed a 73.3- and 35.7-fold lower mRNA concentration on days 2 and 4, respectively; while, with 1μg/L Ag-NPs, there was an 8.0-fold lower mRNA concentration after a 2-day exposure. In contrast, exposure of the fish to the high dosage of $AgNO_3$ led to an induction in the TF mRNA level, about 3.8-fold on Day 2. The other exposure times tested and the lower $AgNO_3$ concentration all showed

some repression (seen during a 1-day exposure) or no significant change in the TF expression levels (Yun Ju Chae et al. 2007).

Acute and sub-lethal effects in juvenile Atlantic salmon exposed to low µg/L concentrations of Ag nanoparticles

A number of studies on aquatic organisms show that the water chemistry is a crucial factor governing toxicity of Ag-NP. Specifically, particle agglomeration can change the size of NPs, and this is strongly dependent on factors such as dissolved organic carbon (DOC), pH and ionic strength (Gao et al. 2009). These factors, as well as varying NP sources, may further complicate inter-study comparisons of Ag-NP toxicity. The purpose of this work was to study the effects of Ag-NP exposure to fish at low µg/L concentrations.

Two Ag-NP preparations were tested: a commercially available, 20 mg/L colloidal silver suspension and an in-house laboratory synthesised Ag-NP solution.

The biological response showed that elevated gill Ag levels were observed in all exposure groups, with the exception of the 1 µg/L commercial AgNP suspension thus verifying that Ag-NP or Ag ions were accumulating or being deposited/adsorbed on the gill epithelial structures. However, since natural Ag(I) complexes may also deposit/adsorb to gill surfaces, the high Ag accumulation reported here could potentially indicate deposition/adsorption or increased bioavailability of silver from the low ion (soft) lake water. No significant differences were observed in the concentration of gill Ag between exposure groups.

High (73%) mortality was found in the group exposed to 100 µg/L commercial Ag-NP (NP1) after 48 h exposure. Gill histopathology of all (n = 4) the remaining live fish in this group demonstrated widespread cell degeneration and necrosis of the epithelial lining of secondary gill arches. The changes observed were themselves enough to explain the high mortality within this group. The current findings therefore represent one of the first observations of detrimental gill damage in fish exposed to Ag-NP. The gills from fish exposed to 20 µg/L Ag-NP or Ag(I) were not affected in the present study, thus suggesting a no observed effect concentration (NOEC) for acutely toxic effect of 20 µg/L for both Ag-NP and Ag(I) ions.

As sensitive biomarkers of general stress, metal exposure and osmoregulatory toxic effect respectively, heat shock protein 70 (HSP 70), metallothionein A (MT-A) and Na/K ATPase were chosen for quantitative gene expression analysis by qPCR shows that HSP 70 was induced at 100 µg/L of both NP1 and NP2 preparations as well as to 20 µg/L Ag(I) ions, indicating a general stress response at 20 µg/L for the commercial Ag-NP suspension as well as 20 µg/L Ag(I) ions. Results in the present experiment showed significant gene down-regulation of Na/K ATPase at exposure concentrations of 20 µg/L for NP1 and ionic Ag(I) as well as 100 µg/L NP1 and NP2. The gene down-regulation was similar between fish exposed to of Ag(I) ions or either AgNP preparations. Hence both Ag-NPs and Ag(I) ions could impair the osmoregulatory capacity potentially by specific inhibition of Na/KATPase, albeit additional effects caused by AgNPs clogging the gill epithelial surface could not be ruled out as a contributing factor (Farmen et al. 2009).

Histopathological studies and oxidative stress of synthesized silver nanoparticles in Mozambique tilapia (*Oreochromis mossambicus*)

In the acute toxicity test, adult fish (8 per group) were maintained in 10 L glass aquaria, and exposed to a graded series of Ag- NPs at 25, 50 and 75 mg/L for eight days.

Histopathology studies

After dissecting the Ag-NPs media exposed fishes, small pieces of the liver, skin and gill

tissues were fixed in neutral buffered formalin. The histopathology slides suggested the presence of the Ag NPs in the *O. mossambicus* tissues. In the liver tissues of exposed fish to Ag-NPs at 50 mg/L showed cloudy swelling of hepatocytes, congestion, vacuolar degeneration, karyolysis, karyohexis, dilation of sinusoids and nuclear hypertrophy Mild congestion of blood vessels were seen in the gill primary lamellae at 50 mg/L exposure level, showed a fusion of primary lamellae and marked hyperplasia of the branchial arch was evident at 50 mg/L concentration.

Superoxide dismutase levels (SOD) and catalase (CAT) and peroxidase (POD) analysis

Exposure to 25 mg/L Ag-NPs, the SOD activities of different tissues were stimulated and showed a remarkable increase, which might be due to the synthesis of new enzymes or the enhancement of pre-existing enzyme levels under lower concentrations. CAT and POD are the key enzymes in antioxidant defense systems to convert the resulting free radicals H_2O_2 to water and oxygen. In the present study, CAT and POD activities in different tissues of Tilapia fish at different Ag-NPs concentrations and an exposure at different time, respectively. The CAT activity of different tissues showed a slight decrease up to day 2 and then a remarkable elevation was observed at 50 mg/L. In addition, the CAT and POD activities in the liver were 2–3 folds and 5–10 folds of NPs accumulation in the gill and the skin at the same concentrations of experimental media exposure, respectively (Rajakumar et al. 2012).

Conclusion

The use of nanomaterials and their potential environmental and human health risks (Andujar et al. 2009; Helfenstein et al. 2008) are of increasing concern and social debate (Dean, 2009; Feder, 2006); (RS/RAE, 2004). Toxicity data for fish exposed to Ag-

NP vary considerably, reflecting species sensitivity differences, characteristics of the different Ag-NP formulations and the water chemistry conditions used in the different studies. In conclusion, silver nanoparticles at different μg/L concentrations result in numerous physiological and biochemical responses indicative of silver toxicity. Histopathological studies have been conducted to help understand causal relationships between dose dependent response and various biological responses.

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