Differential toxicity of silver and titanium dioxide nanoparticles on *Drosophila melanogaster* development, reproductive effort, and viability: Size, coatings and antioxidants matter

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**Abstract**

Silver and titanium dioxide nanoparticles are known to induce oxidative stress in vitro and in vivo. Here we test if they impact development, mating success, and survivorship in *Drosophila melanogaster*, and if so, if these effects are reversible by antioxidants. Ingestion of nanotitanium dioxide during the larval stage of the life cycle showed no effects on development or survivorship, up to doses of 200 μg mL⁻¹. Conversely, ingestion of nanosilver had major dose, size, and coating-dependent effects on each of these aspects of life history. Each of these effects was partially or fully reversible by vitamin C. Larvae growing on nanosilver supplemented with vitamin C showed a greater than twofold increase in survivorship compared to flies reared on nanosilver alone, and a threefold increase in mating success. Vitamin C also rescued cuticular and pigmentation defects in nanosilver fed flies. Biochemical assays of superoxide dismutase and glutathione show these markers respond to nanotitanium dioxide and nanosilver induced oxidative stress, and this response is reduced by vitamin C. These results indicate that life history effects of nanosilver ingestion result from oxidative stress, and suggest antioxidants as a potential remediation for nanosilver toxicity. Conversely, the lack of nanotitanium dioxide life history toxicity shows that oxidative stress does not necessarily result in whole organism effects, and argues that nanoparticle toxicity needs to be examined at different levels of biological organization.

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1. Introduction

Nanoparticle-based technologies are a rapidly growing facet of our industrial, medical and environmental economies. Their toxicological properties, different from bulk form, have been subject to intensive research (Oberdörster et al., 2005; Nel et al., 2006; Rivera Gil et al., 2010). Silver and titanium dioxide nanoparticles, used in clothing, food industry, paints, cosmetics, electronics, coatings and medical products, are of particular concern because they are two of the fastest growing product categories in the nanotechnology industry (Cheng et al., 2004; Cohen et al., 2007; Lee et al., 2007; Vigneshwaran et al., 2007).

Oxidative stress is an emerging, general mechanism underlying nanoparticle toxicity (Nel et al., 2006; Mocan et al., 2010). Previous work in our lab (Ahamed et al., 2010) finds that nanosilver ingestion in *Drosophila* larvae activates oxidative stress pathways, including superoxide dismutase (SOD), catalase, caspase-3 and caspase-9, glutathione and malondialdehyde (MDA), a product of lipid peroxidation. Titanium dioxide is best known for reactive oxygen species (ROS) production via photoactivation (Hirakawa et al., 2004), however, studies find ROS production from both rutile and anatase in the absence of photoactivation (Gurr et al., 2005; Braydich-Stolle et al., 2009).

Yet to be determined is whether oxidative stress has effects on the whole organism, in terms of development, reproduction, and survival. The lowest level of cellular response to oxidative stress entails the induction of antioxidant molecules and detoxification enzymes via Nrf-2 activation (Xiao et al., 2003). Thus, acute exposures may have only transient cellular effects that do not impact the health of the organism. Conversely, sustained ROS production can lead to inflammation, circulatory problems, and DNA damage that could ultimately impact the life history of the organism through effects on development, viability, and/or reproductive effort.

There are few *in vivo* studies on life history effects of chronic titanium dioxide and silver nanoparticle exposure, almost all in aquatic organisms (reviewed in Menard et al., 2011). Such studies are needed, both to determine the potential for life history toxicity in mammalian models, and the potential for environmental damage through their extensive commercial production and consumer...
use. *Drosophila melanogaster* provides a relevant model for investigating human health, as counterparts of genes responsible for more than 700 different human genetic diseases including neurological, immunological, cardiovascular, auditory, visual, developmental and metabolic disorders are found in *Drosophila* (Reiter et al., 2001; Koh et al., 2006; Wolf et al., 2006; Khurana et al., 2006). Moreover, the Nrf-2 gene and its function are conserved in *Drosophila*, making it specifically relevant to model oxidative stress responses (Sykiotis and Bohmann, 2008). The cost effectiveness, experimental flexibility, and short generation time of *Drosophila* permit rapid *in vivo* assessment of the vast number of nanoparticles being produced, including life history effects of chronic exposure.

We find that nanosilver ingestion had negative, dose-dependent effects on survivorship, mating success and development, with smaller, uncoated particles showing the greatest toxicity. Conversely, titanium dioxide had no effect on fly life history. Vitamin C ingestion partially or fully reversed each of these life history effects of nanosilver ingestion, and reduced the response of oxidative stress markers SOD and glutathione (GSH). While antioxidant reversal of nanoparticle induced stress has been observed *in vitro* (Kim et al., 2009; Akhtar et al., 2010; Sharma et al., 2010; Foldbjerg et al., 2011), this is the first *in vivo* study that shows nanoparticle toxicity can be alleviated by antioxidants. The *in vivo* reversal of each facet of nanosilver toxicity indicates that the primary mechanism of nanosilver toxicity is oxidative stress. The lack of nanotitanium dioxide life history toxicity provides a caution that biochemical toxicity does not necessarily extrapolate to whole organism toxicity.

2. Experimental procedures

2.1. Nanoparticles used in this study

Uncoated and poly-saccharide-coated 10 nm and 60 nm silver particles were generously provided by Dr. Dan Goia (Clarkson University Center for Advanced Materials Processing, Potsdam, NY). The coated silver nanoparticles were synthesized by the reduction of silver ions in a solution of a poly-saccharide (acacia gum), which leads to surface coating. Nanoparticles were diluted from bulk concentrations to 1 mg mL$^{-1}$ working stock concentrations and sonicated for 1 min at 35–40 W to aid in mixing and forming a homogeneous dispersion.

2.2. Nanoparticle characterization

Transmission electron microscopy (TEM) characterization was performed to determine nanoparticle size and morphology using a Hitachi H-7600 tungsten-tip instrument at an accelerating voltage of 100 kV. Nanoparticles were examined after deposition of nanoparticle suspensions onto carbon film-coated Cu TEM grids. The AMT software for the digital TEM camera was calibrated for size measurements of the nanoparticles. Information on mean size and SD was calculated using point to point method (Murdock et al., 2008).

Dynamic light scattering (DLS) and laser Doppler velocimetry (LDV) were used for characterization of hydrodynamic size and zeta potential ($\zeta$) of nanoparticles suspended in H$_2$O, performed...
on a Malvern Instruments Zetasizer Nano-ZS instrument as described by Murdock et al. (2008).

2.3. Fly husbandry

OreRS flies were obtained from the Bloomington Stock Center, Bloomington, IN and reared on standard cornmeal–malt–yeast medium (Bloomington Stock Center recipe) at 25 °C. Nanoparticles and vitamin C were added to the media while cooling and decanted into 60 mm × 15 mm petri plates for larval feeding experiments. Control plates consisted of 20 mL standard Drosophila cornmeal media. In treatment lines, standard medium was supplemented with nanoparticles resulting in final suspensions from 5 μg to 200 μg nanoparticles per mL of medium, depending on the experiment. In antioxidant experiments, medium was supplemented with ascorbic acid (vitamin C) or vitamin C palmitate (a lipid-soluble vitamin C) to a final concentration of 50 mM.

2.4. Survivorship assay

Drosophila embryos were laid over a 2 h time period on control medium, 50 of which were collected and moved to control or treatment plates for the assay. Seven plates of 50 embryos each were used for each treatment. Percent survivorship was calculated as the number of embryos that pupated divided by the total number of embryos. The time from first larval instar hatch to pupation was also recorded. Adult phenotypes were scored following eclosion for cuticle maturation and melanization, two features found to vary with nanosilver dose.

2.5. Mating success assay

Virgin male and female flies that survived ingestion of 60 nm uncoated silver during the larval stage were mated in pairs. Twenty pairs were used for each treatment. The number of successful matings (those resulting in offspring) and number of progeny produced were recorded and compared to control lines (mated pairs not exposed to nanosilver during larval development).

2.6. Biochemical assays

2.6.1. Reagents

Reduced nicotinamide adenine dinucleotide (NADH), reduced glutathione (GSH), 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB), nitrobluetetrazolium (NBT), and phenazine methosulphate (PMS) were purchased from Sigma–Aldrich, MO. All other chemicals used were of the highest purity available from commercial sources.

2.6.2. Preparation of crude extracts

For glutathione and superoxide dismutase assays, 4 d old third instar larvae were exposed for 24 h on control, 50 μg mL⁻¹ Ag NP, 50 μg mL⁻¹ TiO₂, or 50 μg mL⁻¹ Ag NP supplemented with 50 nM vitamin C. Treated larvae were homogenized in 0.1 M sodium phosphate buffer (pH 7.4) containing 0.15 M KCl. Following centrifugation (2300 g for 15 min at 4 °C), total protein of the supernatant was determined using a BCA protein assay kit (Pierce Biotechnology, Rockford, IL). The supernatant (crude extract) was maintained on ice for use in biochemical assays.

Fig. 2. Characterization of silver nanoparticles. (A and B) Represents the TEM characterization of 60 nm uncoated Ag NPs. A total of 130 silver nanoparticles were measured by TEM for size distribution. (A) Depicts the morphology of silver nanoparticles and (B) represents frequency of size distribution of Ag NPs. TEM mean ± SD of Ag NPs was 60.27 ± 11.74 nm. (C and D) Depicts DLS and LDV characterization of 60 nm uncoated Ag NPs. (C) Represents the Ag NPs diameter in water suspension (192 nm) and (D) depicts the zeta potential (~29.4 mV).
2.6.3. Glutathione

GSH level was quantified using Ellman’s reagent (Ellman, 1959). Briefly, a mixture of 0.25 mL of 1 mg mL\(^{-1}\) crude extract and 0.9 mL of 5% TCA was centrifuged (2300g for 15 min at 37 °C). Then 0.5 mL of supernatant added into 1.5 mL of 0.01% DTBN and the reaction was monitored at 412 nm. The amount of GSH was expressed in terms of nmol mg\(^{-1}\) protein.

2.6.4. Superoxide dismutase

SOD activity was determined employing a modified version (Kakkar et al., 1984) of the method described earlier (Nishikimi et al., 1972). Briefly, at room temperature the reaction was initiated by adding 0.2 mL of a 780 μM NADH solution into a reaction mixture containing 1.25 mL of 0.052 M sodium pyrophosphate buffer (pH 8.0), 0.1 mL of 186 μM PMS, 0.3 mL of 300 μM NBT, 0.1 mL of distilled water and 50 μL of 1 mg mL\(^{-1}\) crude extract (total volume of 2.0 mL). The reaction was stopped after 1.5 min at room temperature by adding 1.0 mL acetic acid and then 4.0 mL of n-butanol. Following centrifugation (2300g for 15 min at room temperature) the color intensity of chromogen in butanol was measured at 560 nm. A reaction mixture devoid of enzyme served as a control. One unit of enzyme activity is defined as the enzyme concentration required to inhibit chromogen production by 50% in 1 min at room temperature and specific activity expressed as units min\(^{-1}\) mg\(^{-1}\) protein.

2.7. Statistical analysis

The mean and standard deviation of percent survivorship, percent mating success, and time to pupation were calculated and statistically analyzed using an exact binomial test (Goldstein, 1954). Best-fit lines for each treatment were generated using linear regression, and used to determine LD\(_{50}\) (survivorship) or ED\(_{50}\) (mating success). LD\(_{50}\) and ED\(_{50}\) values and biochemical assays were compared to controls using Student’s \(t\)-test (\(\alpha = 0.05\)).

3. Results

3.1. Nanoparticle characterization

Silver 10 nm coated particles were previously characterized by DLS and TEM (Ahamed et al., 2010) and show good dispersion and uniform size. TEM analyses show that all nanoparticles tested are uniform in size (Figs. 1–4). DLS and zeta potential analyses indicate the particle suspensions are relatively stable and form agglomerates in water.

3.2. Survivorship is reduced and developmental time extended by nanosilver ingestion

Nanosilver ingestion had major, dose-dependent effects on survivorship to pupal stage (Fig. 5). Uncoated particles were more toxic than coated particles, and smaller particles were more toxic than larger particles. Time to pupation was slowed by nanosilver ingestion in a dose-dependent manner (Fig. 6). This effect plateaus around 180 h, apparently extension of the pre-adult lifecycle is not viable beyond this time under our experimental conditions. Neither survivorship nor time to pupation was affected by nanotitanium dioxide (rutile) ingestion at doses up to 200 μg mL\(^{-1}\).

Fig. 3. Characterization of silver nanoparticles. (A and B) Represents the TEM characterization of 60 nm poly-saccharide coated Ag NPs. A total of 139 silver nanoparticles were measured by TEM for size distribution. (A) Depicts the morphology of silver nanoparticles and (B) represents frequency of size distribution of Ag NPs. TEM mean ± SD of Ag NPs was 66.54 ± 15.7 nm. (C and D) Depicts DLS and LDV characterization of 60 nm poly-saccharide coated Ag NPs. (C) Represents the Ag NPs diameter in water suspension (93 nm) and (D) depicts the zeta potential (−32.7 mV).
Fig. 4. Characterization of titanium dioxide nanoparticles. (A and B) Represents the TEM characterization of titanium oxide NPs. A total of 200 titanium dioxide NPs were measured by TEM for size distribution. (A) Depicts the morphology of titanium dioxide NPs and (B) represents frequency of size distribution of titanium dioxide NPs. TEM mean ± SD of titanium dioxide NPs length was 38.22 ± 12.14 nm and width was 13.53 ± 3.54 nm. (C and D) Depicts DLS and LDV characterization of titanium dioxide NPs. (C) Represents the titanium dioxide NPs diameter in water suspension (357 nm) and (D) depicts the zeta potential (−25.5 mV).

Fig. 5. Mean and SD percent survivorship for flies fed different concentrations of nanotitanium dioxide and nanosilver during larval development. Treatment line survivorship is normalized to control lines, which are set at 100% survivorship. LD_{50} determined from best-fit line for each treatment generated by linear regression (LD_{50} μg mL^{-1}, R^2): 10 nm Ag coated (32.4, 0.96), 10 nm Ag uncoated (31.0, 0.95), 60 nm Ag coated (47.4, 0.98), 60 nm Ag uncoated (31.2, 0.96). These LD_{50} values are statistically different from each other (t-test, p < 0.05) except 10 nm coated vs. 60 nm uncoated (p = 0.10). Nanosilver ingestion had dose-, size-, and coating-dependent effects on survivorship. Conversely, nanotitanium dioxide had no effect on survivorship (LD_{50} 763 μg mL^{-1}, R^2 = 0.99) at doses up to 200 μg mL^{-1} (data point not shown). N = 350 embryos/treatment.
3.3. Mating success is reduced by nanosilver ingestion

Nanosilver ingestion during the larval stage reduced adult mating success (Fig. 7). Lower doses of nanosilver were sufficient to disrupt reproduction compared to viability; ED$_{50}$ (a dose that results in 50% viable matings compared to control) for uncoated 60 nm silver was 19.6 $\mu$g mL$^{-1}$ ($R^2 = 0.90$), about 2/3 that of LD$_{50}$ for 60 nm uncoated Ag. Many nanosilver-treated flies did not produce offspring, and those that did averaged fewer than half the progeny of control lines.

3.4. Nanosilver ingestion impairs adult cuticle development and melanization

Nanosilver ingestion during the larval stage resulted in cuticular and melanization defects in adults (Fig. 8). Flies that survived nanosilver ingestion had a soft, unpigmented cuticle. No such effect was observed in nanotitanium dioxide-fed flies. As epidermal pigments are secreted by the cuticle (Wright, 1987), the cuticle defect is likely the root cause of these phenotypes.

3.5. Vitamin C ingestion reverses toxic effects of silver ingestion

Previous research finds that nanosilver ingestion causes oxidative stress (Ahamed et al., 2010). This suggests the potential for toxicity reversal through antioxidant treatment, which we verify here.

Larvae growing on 30 $\mu$g mL$^{-1}$ 60 nm uncoated silver supplemented with 50 mM vitamin C or vitamin C palmitate showed greater than twofold survivorship and threefold mating success compared to flies reared on nanosilver alone (Fig. 9). Time to pupation was also reduced significantly ($t$-test), from 158.3 ± 14.2 for nanosilver alone, to 151.0 ± 18.7 ($H_0$: vitamin C < Ag; $p = 0.003$) and 150.0 ± 14.53 ($H_0$: vit. C palmitate < Ag; $p < 0.001$). Nanosilver ingestion resulted in...
Fig. 8. Larval nanosilver ingestion shows dose-dependent effects on adult melanization and cuticular development. The fly in Panel B was fed 30 µg mL⁻¹ Ag uncoated during larval development, and shows a soft adult cuticle lacking pigmentation compared to the control in Panel A. The cuticular and melanization phenotype are rescued by co-ingestion with vitamin C (Panel C, 30 µg mL⁻¹ Ag uncoated + 50 mM vitamin C) and vitamin C palmitate (Panel D, 30 µg mL⁻¹ Ag uncoated + 50 mM vitamin C palmitate).

Fig. 9. The toxic effects of nanosilver ingestion on survivorship and reproductive effort are reversed by vitamin C. Mean and SD percent survivorship and mating pair success are presented for larvae reared on standard medium (negative control), 30 µg mL⁻¹ 60 nm uncoated nanosilver, 30 µg mL⁻¹ 60 nm uncoated nanosilver + 50 mM vitamin C, and 30 µg mL⁻¹ 60 nm uncoated nanosilver + 50 mM vitamin C palmitate. Survivorship comparisons between control and each treatment line are significantly different (t-test, p < 0.01). Survivorship comparisons between nanosilver and nanosilver + vitamin C treatments are significantly different (t-test, p < 0.001). Mating success comparisons between control and each treatment line are significantly different (t-test, p < 0.01), with the exception of vitamin C (t-test, p = 0.34). Mating success comparisons between nanosilver and nanosilver + vitamin C treatments are significantly different (t-test, p < 0.001).

Table 1
Biochemical assays.

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<th>Treatment</th>
<th>SOD assay</th>
<th>GSH assay</th>
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<td></td>
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induced cuticular and melanization defects were also reversed by co-ingestion of vitamin C (Fig. 8c and d).

Larvae growing on 30 µg mL⁻¹ 60 nm uncoated silver supplemented with 50 mM vitamin C showed reduced levels of superoxide dismutase (SOD) and increased, though not statistically significant, levels of glutathione (GSH) compared to flies reared on nanosilver alone (Table 1). This provides a biochemical mechanism whereby vitamin C ingestion can alleviate the effects on nanosilver on the life history traits examined here. Nanotitanium dioxide had comparable effects to nanosilver on SOD activity; but unlike nanosilver, had no effect on GSH levels.

4. Discussion

We show that nanoparticle induced oxidative stress can result in severe life history effects. However, as in the case of TiO₂, oxidative stress does not necessarily affect life history. Both biochemical and life history studies are needed to fully characterize nanoparticle toxicity.

Both nanotitanium dioxide and nanosilver have been shown to activate oxidative stress, DNA, and mitochondrial damage biochemical pathways (Hirakawa et al., 2004; Hussain et al., 2005; Ahamed et al., 2008, 2010; Jemec et al., 2008; Wang et al., 2008; Sharma, 2009; Jin et al., in press). We find that these activations are not necessarily toxic to the whole organism. Nanosilver has major, negative effects on each facet of fly life history assayed in this study. Conversely, nanotitanium dioxide had no effect on survivorship, developmental rate, or adult cuticle phenotype. This shows that nanoparticles must be assayed at different levels of biological organization to determine their full toxicity profile.

Differences in silver and titanium dioxide life history toxicity may simply result from nanosilver showing greater biochemical toxicity. Although the ranges of particle variables relevant to toxicity in the aforementioned studies (different crystal structures, sizes, and shapes) complicate comparisons between nanosilver and nanotitanium dioxide, nanosilver has been shown to induce greater levels of oxidative stress than nanotitanium dioxide, including in a head-to-head study of silver and titanium dioxide nanoparticles comparable to those used in this study (Hussain et al., 2005). While nanotitanium dioxide and nanosilver had comparable effects on SOD levels, nanosilver had a much greater impact on GSH levels than nanotitanium dioxide. GSH is the first line of defense against oxidative stress, its depletion in nanosilver-fed flies could underlie its greater life history toxicity.

Each facet of nanosilver toxicity is partially or wholly remediatably mediated by vitamin C. This indicates that oxidative stress is the major contributor to nanosilver toxicity at each level of organization. In Drosophila, oxidative stress pathways have dual function, they remediate oxidative stress but are also targets of the hormone ecdysone, which directs progression through larval stages of molting and pupal metamorphosis. Their activation in response to oxidative stress could disrupt the timing of these lifecycle events, a possible mechanism underlying the developmental phenotypes observed in treated flies. Such dual roles must be considered in evaluating biochemical toxicity in mammalian models. Moreover, oxidative stress may not be the only mechanism underlying nanoparticle toxicity, it is also possible that other mechanisms additional to oxidative stress are important to nanoparticle life history toxicity and underlie the different toxicity of nanotitanium vs. nanosilver.

Both aqueous and lipid-soluble forms of vitamin C reversed the effects of nanosilver ingestion. Two mechanisms through which vitamin C can act to reverse nanosilver toxicity are: (1) directly through reduction of reactive oxygen species (superoxide ion O₂⁻, hydroxyl radical OH⁻, and/or hydrogen peroxide H₂O₂) and/or (2) through the chelation of silver ions released by silver nanoparticles. Foldbjerg et al. (2011) show in vitro reduction of ROS in human lung cancer cell lines exposed to nanosilver when cells are pretreated with the antioxidant N-acetyl-cysteine, however, this could result from antioxidant effects on either the nanosilver particle or the ion. Preliminary results in our lab find that the polysaccharide-coated nanosilver tested here release silver ions at a greater rate than uncoated nanosilver. Yet uncoated silver particles had greater toxicity than the same size coated particles, indicating silver ion release at most makes a small contribution to toxicity. A direct comparison of the efficacy of vitamin C on coated vs. uncoated nanosilver, with a silver ion control would help determine the mechanism of vitamin C reversal of nanosilver toxicity, and more generally nanosilver toxicity.

In addition to coating, size and agglomeration state are also relevant to toxicity. Though coated particles show less agglomeration in our in vitro measurements, resulting in a relatively higher reactive surface area for the generation of ROS, they are less toxic than the same sized uncoated particle. The nature of the surface is clearly more relevant to toxicity than the amount of surface area available. Although the smaller 10 nm silver particles showed somewhat greater toxicity than equivalently coated 60 nm particles, the smaller particles provide six times more reactive surface area per unit mass, a much greater difference than found in their toxicity, in particular as smaller particles show less agglomeration in in vivo measurements. The interaction between coating, size and agglomeration state in toxicity is clearly complex and in need of further study.

The lack of 1:1 correspondence between biochemical and life history aspects of nanoparticle toxicity strongly indicates a need to research the impact of biochemical stress on the whole organism, in chronic toxicity models capable of testing for life history effects. Studies that focus on the relationship between in vitro and in vivo effects of oxidative stress may determine threshold values of biochemical ROS generation that result in life history toxicity, which would be relevant to setting exposure standards in industrial and commercial use.

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References


