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Oxidative stress and genotoxic effects of diamond nanoparticles

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ABSTRACT

Due to the unique and useful properties of nanodiamonds (ND), their production and use is rapidly increasing. Thus, more of these particles will be released into the environment and organisms will inevitably be exposed to them. The current knowledge about the toxicity of ND, especially in vivo toxicity, is fragmentary. In this study, the toxicity of nanodiamonds was assessed in *Acheta domesticus* following chronic exposure to different nominal concentrations of ND (20 and 200 $\mu g\ g^{-1}$ food) administrated in food for the entire lifespan. The activity of oxidative stress enzymes (catalase, glutathione peroxidase), total antioxidant capacity, as well as the level of heat shock protein were determined. A significant increase in all of the measured parameters was observed after seven weeks of exposure in individuals exposed to higher concentrations of ND (200 $\mu g\ g^{-1}$ food). In animals exposed to lower concentrations of ND (20 $\mu g\ g^{-1}$ food), there were few significant changes to these parameters. Analysis of DNA damage performed after fourteen weeks using the comet assay revealed DNA instabilities in the insects, especially the ones that had been exposed to the higher doses of ND. These findings may suggest that the toxicity of ND is concentration dependent. While high doses interact in a toxic manner, trace amounts, which are more likely in the environment, might be safe for organisms. Extreme caution should be taken when handling nanodiamonds.

1. Introduction

Nanomaterials are used in various branches of industry and science due to their unique and useful properties (Yuan et al., 2010). The presence of nanoparticles in commercially available products is increasingly common (Lewinski et al., 2008). Their production and use are growing rapidly (Yuan et al., 2010). It is projected that the production of nanoparticles will increase to 58,000 t by 2020, compared to the estimated 2300 t being produced nowadays (Maynard, 2006a, 2006b). However, the consequences of advancements in nanotechnology will result in the increased penetration of these particles into the environment, which may have potentially harmful effects on various organisms. Exposure of animals to nanoparticles is inevitable, but knowledge about their toxic effects is still limited (Maynard, 2006a, 2006b). While the number of nanoparticle types and their applications are continuously increasing, studies on their effects and potential toxicity are few. Consequently, nanotoxicology research is now gaining attention (Lewinski et al., 2008).

Nanodiamonds (ND) are allotropes of carbon that were discovered several decades ago (Baidakova and Vul, 2007). They have gained a high level of importance because, among various other nanomaterials, they have extreme chemical inertness, optical transparency, exceptional hardness and good biocompatibility (Huang et al., 2007). Currently, hundreds of tons of ND are produced in various areas worldwide every year. They can be used in electrochemical coatings, polymer compositions, antifriction coatings, polishes, lubricants, biosensors, imaging probes, implants and also as drug carriers (Yuan et al., 2010). A distinct feature of nanodiamonds, compared to carbon nanotubes and other graphitic nanoparticles, is that after attaching various groups to their surface, it is possible to produce quite sophisticated surface functionalization without compromising the useful properties of the diamond core (Mochalin et al., 2011). One example of their potential impact can be their interaction with food molecules. The fate of ND after getting into the body via food or whether they can cause adverse effects is not known. Moreover, present knowledge about the *in vivo* toxicity of ND is very limited. Very little research is focused on the ecotoxicological aspect of ND; the vast majority is concentrated on the rapid and usually unnatural, administration of nanoparticles. Such studies are primarily conducted on specific cell lines. When organisms are used in research, the nanoparticles are

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usually administered by injection. Cid et al. (2015) reported cell damage and an increase in the activity of oxidative stress enzymes, as well as lipid peroxidation in *Corbicula fluminea* following exposure to different doses of ND over 14 days. Similar results were reported by Yuan et al. (2010) in a 28-day assay in which ND were injected intravenously. Furthermore, ND are able to accumulate in the organs of mice (especially in the liver, lungs and spleen). Mendonça et al. (2011) assessed the potential toxicity of ND suspended in water on *Daphnia magna* and reported an increase in mortality and the inhibition of reproduction. Moreover, diamond nanoparticles adhered to the exoskeleton surface and were accumulated within the gastrointestinal tract.

A better understanding of how ND interact with cells, tissues and organs is essential to ensure the safe use of ND. Knowledge about the *in vivo* toxicity of ND is extremely scarce. It is suspected that the underlying mechanisms of nanoparticle toxicity are oxidative stress, genotoxicity, inflammation and immunotoxicity. There are indications that the toxicity of nanodiamonds is primarily associated with the induction of the first two (Magdolenova et al., 2014).

The aims of this work were to characterize the toxicity of nanodiamonds after exposure to different concentrations that were administered with food during the entire life cycle of *Acheta domesticus*. DNA damage, DNA regeneration at consecutive time points (0, 5, 15, 30 min after the induction of damage using H_2O_2), total antioxidant capacity (TAC), the activity of selected antioxidant enzymes (CAT, GSTPx) and the level of heat shock protein (HSP 70) were analyzed in selected organs.

2. Material and methods

2.1. House cricket

A. domesticus (Gryllidae, Orthoptera) is native to Southwestern Asia, but in reality it occurs all around the world. The house cricket is used as a model organism in physiological and toxicological research quite often (Szelei et al., 2011). The species is omnivorous and easy to breed; its life cycle lasts about 3–4 months. The animals used in the experiment were derived from a laboratory stock population maintained in standard conditions at the University of Silesia in Katowice.

2.2. Breeding conditions

Newly hatched insects were caught and randomly divided into the experimental groups. Animals were gently transferred into plastic boxes ($28 \times 20 \times 16$ cm; 50 individuals in each) and bred under standard conditions (temperature: 28.8 ± 0.88 °C; photoperiod L:D 12:12; humidity: 20–45%) with unlimited access to water and food. During the first two weeks of life, the crickets were kept on a standard, uncontaminated diet (Kanisan Q, Sano, Poland). Then, food containing different amounts of ND (0 $\mu g\ g^{-1}$ – control group; 20 $\mu g\ g^{-1}$ or 200 $\mu g\ g^{-1}$ – ND20 and ND200 groups, respectively) was supplied each day until the end of the crickets' life. The nanoparticles were purchased in PlasmaChem (Single Digit NanoDiamonds, aqueous suspension; diamond crystallite size: 3.5–5.2 nm; particle size (DLS): 5–15 nm; PlasmaChem GmbH; Berlin). In addition to the characteristics of the ND that were supplied by the producer, additional analysis of the structure of material used was conducted by our team (see below). The food with nanodiamonds was prepared by grinding the standard food and mixing it with ND dissolved in distilled water. Subsequently, the food was dried for two days in a dryer at 50 °C and then sterilized in a laminar box for 24 h. Food for the control group was prepared in the same manner but without ND. When the insects reached maturity (seven weeks after hatching), six individuals from each group were randomly chosen and prepared for HSP 70 and enzymatic analysis. Individuals for the measurements of DNA damage (five insects from each group) were collected 14 weeks after hatching.

2.3. Characteristics of the nanodiamonds

Samples for TEM observation were prepared by dispersing ethanol suspensions of the material onto lacey carbon-coated copper grids. TEM investigations were performed using a field-emission transmission electron microscope (FEI Titan 80-300 TEM/STEM) with a super twin lens operating at 300 kV. The chemical composition was determined with the same apparatus using Electron Energy Loss Spectroscopy (EELS). Information about the crystal structure of the diamonds came from the database maintained by the Mineralogical Society of America and the Mineralogical Association of Canada (Downs and Hall-Wallace, 2003).

2.4. Histological evaluation of the ultrastructure of the gut epithelium

The midgut isolated from adult specimens of *A. domesticus* of two groups (control group and ND200 group) was fixed in 2.5% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.4 for 1.5 h at 4 °C) and then postfixed in 1% OsO_4 for 1.5 h at room temperature. After dehydration in a graded concentration series of ethanol (50%, 70%, 90%, 95% and 100% for 15 min each) and acetone (15 min), the material was embedded in epoxy resin (Epoxy Embedding Medium Kit, Sigma). Ultrathin sections (70 nm) were cut on a Leica Ultracut UCT25 µltramicrotome. A series of ultrathin sections were stained with uranyl acetate and lead citrate and then were examined using a Hitachi H500 transmission electron microscope at 75 kV.

2.5. Preparation of samples for biochemical analysis

The insects were slightly anesthetized on ice and then the hemolymph, gastrointestinal tract, head, gonads and fat body were collected. The hemolymph was obtained by cutting between the body and the coxa with scissors after which drops of the seeping hemolymph (ca 40 µL) were gathered in Eppendorf tubes filled with 40 µL of an anticoagulant buffer (170 mM NaOH, 186 mM NaCl, 12 mM Na_2EDTA, 45 mM citric acid). Other tissues were homogenized in a 0.1 M phosphate buffer (pH 7.4) after dissection and centrifuged at $15{,}000 \times g$ for 10 min at 4 °C in order to obtain a submitochondrial fraction. Standardization of the methods of analysis for the house cricket tissue was conducted for all of the assayed parameters. The kinetic reaction was also checked. Blank samples (without supernatants) as well as boiled samples were tested to control the non-enzymatic reaction rates. All measurements were performed using a UV–vis spectrometer (TECAN Infinite M200, Austria).

2.6. Total Antioxidant Capacity assay

The Total Antioxidant Capacity (TAC) assay was determined according to Re et al. (1999). The radical of 2,20-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) was prepared by reacting ABTS with potassium persulphate in a sodium phosphate buffer (pH 7.4) for 12–16 h in darkness. The blue-green solution was kept at 20 °C prior to use. Decolorization of ABTS was measured at 734 nm. Results were expressed in terms of the equivalent anti-oxidant capacity to 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and expressed in µmol Trolox per mg protein.