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Cadmium sulfate and CdTe-quantum dots alter DNA repair in zebrafish (*Danio rerio*) liver cells

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ABSTRACT

Increasing use of quantum dots (QDs) makes it necessary to evaluate their toxicological impacts on aquatic organisms, since their contamination of surface water is inevitable. This study compares the genotoxic effects of ionic Cd versus CdTe nanocrystals in zebrafish hepatocytes. After 24 h of $CdSO_4$ or CdTe QD exposure, zebrafish liver (ZFL) cells showed a decreased number of viable cells, an accumulation of Cd, an increased formation of reactive oxygen species (ROS), and an induction of DNA strand breaks. Measured levels of stress defense and DNA repair genes were elevated in both cases. However, removal of bulky DNA adducts by nucleotide excision repair (NER) was inhibited with $CdSO_4$ but not with CdTe QDs. The adverse effects caused by acute exposure of CdTe QDs might be mediated through differing mechanisms than those resulting from ionic cadmium toxicity, and studying the effects of metallic components may be not enough to explain QD toxicities in aquatic organisms.

Introduction

Quantum dots (QDs) are highly photostable fluorescent nanocrystals with electronic and medical applications such as LEDs, diagnostic imaging, and biosensors (Juzenas et al., 2008; Medintz et al., 2005; Suh et al., 2009). It is inevitable that QDs will be released into the environment, where their toxicological parameters are not well defined and their mechanisms of action are unclear. A number of toxicological studies of QDs have been performed in cell lines and bacteria (Chibli et al., 2011; Cho et al., 2007; Clift et al., 2010; Cooper et al., 2010; Dumas et al., 2009, 2010; Mahto et al., 2010; Park et al., 2011); however, to date, none have focused on the effect of QDs on DNA repair.

Since surface waters receive pollutants containing QDs from various sources, such as atmospheric deposition, wastewater effluents (Scown et al., 2010), and leaching from soil, and act as reservoirs for numerous anthropogenic contaminants, it is critical to evaluate the potential impacts of QDs on aquatic organisms (Leigh et al., 2012). In the case of cadmium (Cd)-based QDs, such as cadmium telluride (CdTe), studies of effects on aquatic species are scarce. Several studies in freshwater mussels have reported the impacts of CdTe QDs on Cd bioaccumulation, metallothionein (MT) production, oxidative stress, genotoxicity, and the immune system (Gagné et al., 2008a, 2008b; Peyrot et al., 2009). In rainbow trout hepatocytes, aged CdTe QDs are known to induce MT, DNA damage, lipid peroxidation and heat shock protein 70 kDa (Gagné et al., 2008a, 2008b). In a more recent study in rainbow trout, CdTe QDs altered hepatic gene expression and suppressed immunity in a fashion different than what was observed with cadmium salts ($CdSO_4$) (Gagné et al., 2010).

Toxicity of CdTe QDs has been attributed to a variety of mechanisms, including free cadmium ions present in particle solution, the leaching of Cd^{2+} ions from QD core (Chen et al., 2012; Cho et al., 2007; Gagné et al., 2008a, 2008b; Rzigalinski and Strobl, 2009), and generation of reactive oxygen species (ROS) (Cho et al., 2007; Clift et al., 2010; Gagné et al., 2008a, 2008b). When ROS generation by Cd^{2+} or CdTe QDs overwhelms the cellular antioxidant capacity, it causes oxidative damage to cellular macromolecules via radical reactions with DNA, lipids, and proteins. Oxidative damage to DNA may result in mutagenesis and is associated with carcinogenesis (Powell et al., 2005; Sandrini et al., 2009a). Cadmium has been classified as carcinogenic to humans and animals by the International Agency for Research on Cancer.

Oxidative stress triggers DNA repair mechanisms in cells (Brierley and Martin, 2013). The DNA repair system comprises multiple distinct mechanisms: base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), and non-homologous end joining (NHEJ) (Friedberg, 2003). Cd^{2+} inhibits BER and NER by several mechanisms, leading to a diminished removal of DNA lesions induced by

Abbreviations: QDs, quantum dots; ZFL, zebrafish liver cells; ROS, reactive oxygen species; BER, base excision repair; NER, nucleotide excision repair; MMR, mismatch repair; NHEJ, non-homologous end joining; Cd, cadmium; CdTe, cadmium telluride; MPA, mercaptopropionic acid; TEM, transmission electron microscope; GFAAS, graphite furnace atomic absorption spectrometry; MT, metallothionein; SOD, superoxide dismutase; PCoA, principal coordinate analysis.

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environmental mutagens (Candéias et al., 2010). However, little is known about the effects of CdTe QDs on DNA repair processes, and a question of particular concern is whether QDs have the same effects as Cd^{2+} on NER capacity.

The zebrafish (*Danio rerio*), with a highly conserved and competent DNA repair pathway (Feitsma and Cuppen, 2008; Langheinrich et al., 2002; Sussman, 2007; Zeng et al., 2009), has been used as a vertebrate model organism in studies of environmental toxicology (Stegeman et al., 2010), nanotoxicity (Fako and Furgeson, 2009), and cancer (Feitsma and Cuppen, 2008). In vivo, QDs can be distributed to the liver (Hardman, 2006), which is also a principal target of Cd^{2+} distribution and toxicity (Cambier et al., 2010; Leazer et al., 2002). The zebrafish liver (ZFL) cell line has been extensively applied to study the toxic effects of metal nanoparticles or their elemental components in vitro (Chen and Chan, 2011; Cheuk et al., 2008; Wan et al., 2009), and to investigate the impacts of environmental carcinogens or mutagens on the DNA repair system (Notch and Mayer, 2009a,b; Sandrini et al., 2009a, 2009b).

This study aimed to compare the impacts of $CdSO_4$ and CdTe QDs on cell survival, Cd accumulation, ROS formation, genetic damage, expression of several key DNA repair genes, and overall NER repair capacity in zebrafish hepatocytes in vitro. Soluble Cd^{2+} and CdTe QDs had similar effects on cell survival, ROS formation, and gene expression. Three substantial differences were seen between the two types of exposure: in total Cd accumulation, DNA damage, and DNA adduct formation and repair. Soluble Cd^{2+} led to measurably more DNA damage than non-photoexcited QDs. Alteration in formation and removal of adducts was also seen only with soluble Cd^{2+}. These findings suggest mechanistic differences between toxic effects of soluble Cd^{2+} and Cd-based nanoparticles, and pave the way for future studies.

Materials and methods

Synthesis of CdTe QDs. CdTe QDs were synthesized in octadecene following a published procedure (Kloper et al., 2007). They were solubilized via cap exchange using mercaptopropionic acid (MPA) as described (Dumas et al., 2009) and dissolved in 50 mM borate buffer, pH 9. Images were captured on a Philips CM200 transmission electron microscope (TEM). 100 CdTe QDs were quantified using TEM to establish the distribution and mean of particle sizes. Absorbance spectra were determined on a SpectraMax Plus plate reader and emission spectra on a SpectraMax Gemini (Molecular Devices, Sunnyvale, CA). Distribution of nanoparticle sizes was also exemplified by the narrow width of the emission peak which is also an excellent measure of the distribution (data not shown).

Chemical characterization of $CdSO_4$ and CdTe QDs. A stock solution of 19 μM CdTe QDs in borate buffer or 50 mM $CdSO_4$ (Sigma-Aldrich) was analyzed to characterize Cd content. A portion of the stock solution was diluted to a desired concentration, and then free Cd^{2+} ion concentration was measured using Measure-iT™ lead and cadmium assay kit (Molecular Probes) (Cho et al., 2007; Mahto et al., 2010). To characterize the total Cd concentration in CdTe QDs and $CdSO_4$ stocks, concentrated nitric acid (Fisher Scientific) was added, the sample was digested for 4 h at 70 °C, then subjected to graphite furnace atomic absorption spectrometry (GFAAS, Thermo Electron Corporation) (Gagné et al., 2010).

Cell culture and viability assay. Zebrafish hepatocytes (lineage ZFL, ATCC, CRL-2643) were maintained at 28 °C with LDF media (Notch and Mayer, 2009a). Cell viability was evaluated by the methylthiazolyl diphenyl-tetrazolium bromide (MTT) assay (Chang et al., 2009). Cells were plated in 96-well plates (Costar 3603) at 3×10^4 cells per well with 1 mL medium and allowed to adhere for 24 h. $CdSO_4$ or CdTe QDs were added in 1 mL of medium and cells were then incubated at 28 °C in the dark for 21 h. 10 μL of 5 mg/mL MTT (Affymetrix) in PBS was added to each well and incubated for 3 h, at which point 150 μL of DMSO (Sigma-Aldrich) was added to solubilize formazan. The plates were placed on a shaker for 15 min to fully dissolve the reagent, and then absorbance was quantified at 570 nm and 650 nm with a Synergy HT Multi-Mode Microplate Reader (BioTek). At least four replicates per treatment were analyzed simultaneously and the experiments were repeated in triplicate. The percentage of viable cells relative to control (100% viable) was plotted, and 50% inhibitory concentration (IC-50) was determined from curve fits using GraphPad Prism 5.0 software (Graphpad Software, La Jolla, CA). Besides, ZFL cell viability after different treatments was double-checked by cellular morphology and direct viability count using a hemocytometer under an inverted Olympus microscope.

Cadmium accumulation in cells. For cellular Cd accumulation, we adapted the protocols from previous copper studies in the ZFL cell line (Chen et al., 2011; Sandrini et al., 2009a). Cells were placed in a 6-well plate (Costar 3516) at 6×10^5 cells per well with 2 mL medium and allowed to attach for 24 h at 28 °C in the dark. The culture medium was removed and the attached monolayer of cells was washed with Ca^{2+}- and Mg^{2+}-free PBS. Various concentrations of $CdSO_4$ or CdTe QDs were added in 2 mL of medium. During exposure, cells were kept at 28 °C in the dark. After 24 h, the experimental medium was removed, the monolayer of cells was washed with fresh PBS, and trypsin–EDTA (Invitrogen 25300-062) was added. After trypsinization, cells were collected and centrifuged for 5 min at 400 ×*g*. The cell pellet was washed in 1 mL PBS twice to remove adherent chemicals. 100 μL of resuspended cells was used to determine cell numbers using a hemocytometer. Remaining cells were centrifuged and dried at 60 °C, and then digested in 50 μL concentrated HNO_3 for 24 h until the solution is clear. Cd concentration was measured by GFAAS. Triplicate pseudo-replicates were analyzed simultaneously.

Measurement of soluble Cd^{2+} concentration after CdTe QDs treatment. Soluble Cd^{2+} ion concentration after 24 h CdTe QDs treatment was measured using Measure-iT™ lead and cadmium assay kit as mentioned above. Cells were plated in 96-well culture plates (Costar 3603) at 3×10^4 cells per well with 1 mL of LDF medium (phenol-red free) and allowed to adhere overnight. After attaching, medium was removed and cells were washed with PBS twice. 1 mL of fresh LDF medium was then added containing 100 nM and 500 nM CdTe QDs. After 24 h exposure, media was removed and collected, and then 100 μL of DMSO was added to lyse the remaining cells and samples were collected. 200 μL of working reagent containing Measure-iT Leadmium reagent (Component A) and 20 mL of 1× buffer were added to each well, which contained 20 μL of samples and Cd standards. After 30 s of shaking, luminescence was read at 490 nm and 520 nm to determine the concentration of soluble Cd^{2+} ion using a Synergy 4 HT Multi-Mode Microplate Reader (BioTek). The $[Cd^{2+}]$ in each sample was determined from standard calibration curves. Three replicates of each concentration were analyzed in parallel.

Intracellular reactive oxygen species (ROS) formation. ROS generation was measured using the DCFDA-Cellular Reactive Oxygen Species Detection Assay Kit (Abcam ab113851) with slight modifications. 1× buffer solution was prepared in LDF media (phenol red-free) instead of deionized water as suggested in the protocol. ZFL cells were cultured in media and about 4×10^6 cells were obtained the day before the experiment. The exponentially growing cells were washed, harvested, and seeded on a dark 96-well microplate with 25,000 cells per well, and then allowed to attach overnight at 28 °C. The plate was washed with 1× buffer solution once, and 100 μL/well of DCFDA mix was added to each well and incubated for 45 min at 28 °C in the dark. The plate was washed with 1× buffer solution once, and then cells were exposed to $CdSO_4$ or CdTe QDs. Fluorescence was read (excitation at 485 nm and emission at 535 nm) on a Synergy HT Multi-Mode Microplate Reader (BioTek) at 6 and 24 h