Single-Wall Carbon Nanotube Inhibition of DNA Damage

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ABSTRACT

Ultrasonication is a physical process known to generate abundant levels of oxygen-derived species such as hydroxyl radicals, hydrogen atoms, and hydrogen peroxide. When used to process DNA solutions, these highly reactive hydroxyl radicals can react with DNA to generate strand breaks and a multiplicity of oxidatively modified DNA bases. Oxidatively induced damage to DNA bases has been well-studied and the reaction mechanisms have been resolved. Gas chromatography-mass spectrometry (GC-MS) methodologies have been developed to both qualitatively and quantitatively measure the DNA lesions. Here, we investigated DNA base damage in (ATT)14 and (GT)20 oligomers from ultrasonication in the presence and absence of single-wall carbon nanotubes (SWCNTs). We found that the overall level of DNA damage is reduced in the presence of SWCNTs, particularly for DNA lesions formed by one-electron reduction of intermediate radicals.

Keywords: nanotoxicology, Comet assay, genotoxicity, biomarker, toxicology

INTRODUCTION

Nanoparticles, defined here as any particle that is less than 100 nm in any one dimension, have substantial potential for commercial applications as a result of their unique properties. With the maturation of this field and a greater understanding of the properties of these particles, there is increasing interest in the use of nanoparticles in consumer products. One of the limitations to the widespread commercialization of nanoparticles is their potential human and environmental health effects. For example, there have been recent modeling efforts to estimate the concentrations expected in different environmental matrices in the US and Europe [1-3]. What still needs to be understood is the interactions of nanoparticles with biomolecules.

One highly promising nanoparticle is single-wall carbon nanotubes (SWCNTs) as a result of their high aspect ratio, electronic properties and substantial tensile strength [4, 5]. However, there are contradictory reports on the literature with regards to their potential toxicity [6-9].

One mode of action that is critical for determining how hazardous a chemical is to humans and organisms is genotoxicity, damage to the genetic material of cells or organisms arising from toxicant exposure. This proceedings paper will focus on oxidative damage to DNA given that oxidative damage is one of the most widely acknowledged mechanisms of toxicity caused by nanoparticles [10]. Approaches that have been used to quantify oxidatively induced DNA damage include liquid chromatography/mass spectrometry (LC/M/S) and gas chromatography/mass spectrometry (GC/MS), which have been used to quantify accumulated levels of individual DNA lesions [11-18]. Lesion levels can be quantified by adding known amounts of stable-isotope labeled internal standards, thus yielding data that are traceable to standard reference materials that can be compared among laboratories to ensure the validity of the measurements.

This conference proceeding focuses on a recent study that examined the effects of the presence of SWCNTs on DNA oligomers during ultrasonication [18]. Previous studies have shown that this process damage DNA bases through the production of free radical species and hydrogen peroxide [19-22]. After sonication, we quantified DNA base lesion formations that are derived from reductive [2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) and 4,6-diamino-5-formamidopyrimidine (FapyAde)] and oxidative [(8-hydroxyguanine (8-OH-Gua) and 5-hydroxy-5-methylhydantoin (5-OH-5-MeHyd)] transformation of the initial OH-adduct radicals.

METHOD

The following method description is modified from [18]. Single-wall carbon nanotubes (SWCNTs) (grade S-P95-02-Dry, batch DU1-A001 CoMoCat) were purchased from Southwest Nanotechnologies (Norman, OK). DNA oligomers were from Integrated DNA Technologies (Coralville, IA), and sodium deoxycholate was obtained from Sigma Aldrich (St. Louis, MO). For sonicated DNA samples, 1 mg of DNA was dissolved in 1 mL 0.1 M NaCl solution followed by ultrasonication with a 3 mm probe sonicator in a 1.5 mL microcentrifuge tube at 8 W for 60 min. To obtain DNA-wrapped SWCNTs, 1 mg of DNA was first mixed with 1 mg of SWCNT and ultrasonicated at the same condition. Oligomers $(GT)_{20}$ and $(ATT)_{14}$ were used. To remove the DNA from the SWCNTs, the DNA was then replaced with surfactant by adding 10 µL of 10% sodium deoxycholate solution to the DNA-SWCNT suspension and incubating for a few hours [23]. The samples were loaded into an OptiSeal™ tube (Beckman-Coulter), placed in a vertical angle (90°C) VTi 65.2 rotor (Beckman-Coulter) and centrifuged at 416 000 *g* and 4 °C for 2 h. The supernatant was extracted and was subject to repeated ultracentrifugation under the same conditions until all of the SWCNTs were removed. The remaining clear supernatant, containing only the DNA, was filtered with an Amicon stirred-cell concentrator (Millipore) with MWCO 10 000 cellulose membrane (Millipore) to remove the salt and the surfactant. The process was repeated a few times, and the sample was washed and redispersed in DI water before the analysis. To examine the effect of a hydroxyl radical scavenger, 0.1% DMSO was added into the DNA samples before ultrasonication with and without SWCNTs. For the control experiments, DNA samples without ultrasonication were used.

Gas chromatography/mass spectrometry (GC/MS) with isotope dilution was used to determine the levels of different oxidatively modified bases in treated and untreated $(GT)_{20}$ and $(ATT)_{14}$ oligomers [12-14]. Five independent samples were prepared for every treatment within the study. DNA pellets for the $(GT)_{20}$ samples were washed three times with ice cold 70% ethanol and once with ice cold absolute ethanol. DNA pellets were dried and then solubilized in distilled and deionized water (ddH_2O). The $(ATT)_{14}$ oligomers did not consistently form pellets in 70% ethanol unlike the $(GT)_{20}$ samples. Therefore, the $(ATT)_{14}$ oligomers were washed using ultrafiltration with 3 kDa membranes as follows: the samples were resuspended in 400 µL of ddH_2O by gently shaking at 4 °C for 24 h and then added to microcentrifuge tubes with 3 kDa membranes (Millipore). The samples were centrifuged at 14,000 g for 99 min at 4 °C. Then, an additional 400 µL of ddH_2O was added and the samples were centrifuged for an additional 60 min. This cleaning step was repeated twice. Two hundred µL of ddH_2O was then added to the top of the filter and the DNA was resuspended by sitting at 4 °C overnight (19 h). DNA aliquots of approximately 50 µg were prepared from each sample and stable isotope-labeled analogues of base lesions (8-OH-Gua-$^{15}N_5$, FapyGua-^{13}C,$^{15}N_2$, 5-OH-5-MeHyd-^{13}C,$^{15}N_2$, and Thymine Glycol-d_4 for $(GT)_{20}$ oligomers, and FapyAde-^{13}C,$^{15}N_2$, 5-OH-5-MeHyd-^{13}C,$^{15}N_2$, and Thymine Glycol-d_4 for $(ATT)_{14}$ oligomers) were added to each sample. Samples were dried under vacuum and then stored at 4 °C prior to enzymatic digestion.

For enzymatic digestion, samples were dissolved in a buffer consisting of 50 mmol/L sodium phosphate, 100 mmol/L potassium chloride, 1 mmol/L EDTA and 100 µmol/L dithiothreitol (pH 7.4) by shaking at room temperature for 1 h. To this solution, 2 µg each of *E. coli* Fpg (Trevigen) and *E. coli* Endonuclease (III) (Trevigen) was added and the digestion was carried out at 37 °C for 1 h. Hydrolysis using these enzymes prevents artifactual formation of DNA lesions because it only releases modified bases; consequently, there is no intact DNA nor unmodified base present during the trimethylsilylation step (see below).[14] The digestion was terminated with the addition of ice cold ethanol and the sample was brought to –20 °C. Samples were centrifuged at 14,000 g for 30 min, supernatant fractions containing the excised DNA lesions were added to glass vials, and the solvent was removed by vacuum desiccation. Samples were solubilized in ddH_2O, lyophilized, and then trimethylsilylated using bis(trimethylsilyl)trifluoroacetamide)/1% trimethylchlorosilane in pyridine (120 °C for 30 min). GC/MS measurements were performed as previously described [12]. A 6890N Network GC System coupled with a 5973 Network Mass Selective Detector) (Agilent Technologies, Inc., Rockville, MD) was employed, and a HP-Ultra 2 high-resolution (12.5 m, 0.2 mm i.d.) fused silica capillary column coated with cross-linked 5% phenylmethylsilicone gum phase (film thickness, 0.33 µm) was used for the GC column (Agilent Technologies, Inc., Rockville, MD). Samples were eluted with a temperature programmed ramp from 130 °C to 270 °C (8 °C per min) followed by holding at 280 °C for 5 min. Trimethylsilyl derivatives of DNA lesions and their stable isotope-labeled analogues were detected using electron ionization mass spectrometry in selected-ion-monitoring (SIM) mode. SIM chromatograms were analyzed using the Agilent MassHunter software (Agilent). Quantification of DNA base lesions was determined using the SIM area ratios from the modified base of interest and its labeled analogue in conjunction with the known amount of the labeled analogue added to each sample. FapyGua, 8-OH-Gua, and 5-OH-5-MeHyd in the $(GT)_{20}$ oligomers, and FapyAde and 5-OH-5-MeHyd in the $(ATT)_{14}$ oligomers were identified and quantified.

Significant differences among the measured lesion levels were determined by one-way analysis of variance (ANOVA) with post hoc Dunnett's multiple comparison test ($\alpha = 0.05$) between the control samples and the

experimental samples. GraphPad Prism 5.0 was utilized for statistical analyses.

RESULTS AND DISCUSSION

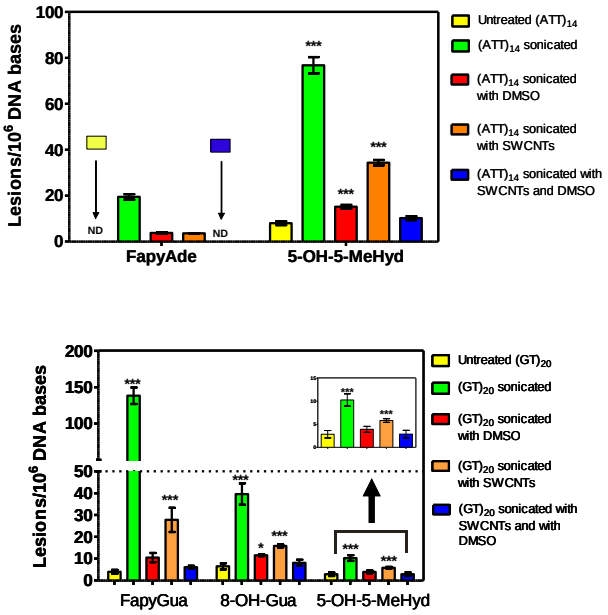

Figure 1. GC/MS DNA damage evaluation of oligomers sonicated in the presence or absence of 0.1% DMSO and SWCNTs. Sonication time was 60 min. Two different oligomers were used: $(ATT)_{14}$ (upper) or $(GT)_{20}$ (lower). The ratio of DNA lesions/10^6 DNA bases represents the mean from five independent samples except for the DMSO only condition for $(GT)_{20}$ oligomer for which four samples were analyzed. The uncertainties represent standard deviations. Statistical analyses based on one-way ANOVA with posthoc Dunnett's multiple comparison test: * p value < 0.05; ** p value < 0.01; *** p value < 0.001. Reprinted with permission from [18].

Table 1. Percentage of the lesion level decrease compared to sonicated samples for $(ATT)_{14}$ oligomers. Reprinted with permission from [18].

	FapyAde	5-OH-5-MeHyd
DMSO alone	– 80.7%	– 80.2%
SWCNT alone	– 81.3%	– 55.5%
DMSO+SWCNT	NA	– 86.7%

Table 2. Percentage of the lesion level decrease compared to sonicated samples for $(GT)_{20}$ oligomers. Reprinted with permission from [18].

	FapyGua	8-OH-Gua	5-OH-5-MeHyd
DMSO alone	– 92.2%	– 70.4%	– 59.7%
SWCNT alone	– 79.9%	– 60.0%	– 43.5%
DMSO+SWCNT	– 95.6%	– 79.6%	– 72.1%

The presence of SWCNTs during ultrasonication decreased the lesion level formation for both oligomers for all of the lesions (see Figure 1 and Tables 1 and 2). Thus, the SWCNTs generally had a protective effect. It is possible that the SWCNTs may have generated radical species and hydrogen peroxide, but the overall decrease in the lesion levels during sonication in the presence of SWCNTs suggests a scavenging effect. The presence of dimethyl sulfoxide (DMSO), a well known scavenger of hydroxyl radicals, during ultrasonication also lead to a substantical decrease in the lesion levels formed. When both DMSO and SWCNTs were present, the lesion levels were decreased more substantially than the presence of either by itself.

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