Tools and Approaches for the Assessment of Nanomaterial Induced Oxidative DNA Damage

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ABSTRACT

Hyphenated mass spectrometry techniques have been employed as one of the primary analytical tools for investigating the effects of ionizing radiation, chemical/biological carcinogens, and oxygen derived free radicals on the induction and subsequent repair of oxidatively-induced DNA damage (DNA lesions) in living systems. The National Institute of Standards and Technology has established a comprehensive research program focused on identifying mechanisms of DNA damage caused by commercially relevant engineered nanoparticles (NPs) using these techniques for the quantification of oxidatively-induced DNA damage. We present an overview of our recent findings from studies on metal (gold) and metal oxide (ultrafine superparamagnetic iron oxide) nanoparticles using isotope dilution liquid chromatography and gas chromatography/mass spectrometry analysis, respectively.

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

INTRODUCTION

In recent years, there has been substantial research interest in nanotechnology as a result of the unique or enhanced properties that many nano-scale particles exhibit. Nanoparticles are defined here as any particle that is less than 100 nm in any one dimension. 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. While research on the properties of nanoparticles for such applications will continue to increase, one of the limitations to the widespread application of nanoparticles is their potential human and environmental health effects. It is inevitable that nanoparticles will be released into the environment, and modeling efforts have begun to estimate the concentrations expected in different environmental matrices in the US and Europe [1-3]. What still needs to be understood is the extent to which these particles pose human or ecological risks resulting from their size-dependant propreties.

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. There are numerous components of genotoxicity such as the potential for gene mutations, chromosomal damage, and oxidative damage to DNA. 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 [4]. Single cell gel electrophoresis (the COMET assay) is the most commonly used test for investigating genotoxicity; however, it is nonspecific and only yields an indication of total DNA damage, including oxidized purine base lesions, oxidized pyrimidine base lesions, abasic sites, and alkali-labile sites in a single number. Alternately, mass spectrometry (MS) based approaches such as liquid chromatography/mass spectrometry (LC/M/S) and gas chromatography/mass spectrometry (GC/MS) have been used to quantify accumulated levels of individual DNA lesions [5-11]. This approach has substantial advantages over the Comet assay such as the potential for mechanistic understandings of the DNA damage process by comparing the relative levels of the different lesions measured. Additionally, 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 two recent studies that determined the ability of nanoparticles to cause oxidatively-induced DNA damage in calf thymus DNA and cells [5, 7]. The potential genotoxic effects of National Institute of Standards and Technology (NIST) reference material (RM) gold nanoparticles (AuNPs) were studied using calf thymus DNA and HepG2 cells, while MCL5 cells were exposed to ultrafine superparamagnetic iron oxide nanoparticles (USPIONs). The AuNP study utilized isotope-dilution LC/MS/MS to quantify 8-hydroxy-2'-deoxyguanosine (8-OH-dG), 8-hydroxy-2'-deoxyadenosine (8-OH-dA), (5'*S*)-8,5'-cyclo-2'-deoxyadenosine (*S*-cdA), and (5'*R*)-8,5'-cyclo-2'-deoxyadenosine (*R*-cdA) lesions, while the iron oxide NP study used GC/MS to measure thymine glycol (TG), 5-hydroxy-5-methylhydantoin (5-OH-5-MeHyd), 2,4-diamino-5-formamidopyrimidine (FapyAde), 2,6-diamino-4-hydroxy-5-

formamidopyrimidine (FapyGua) and 8-hydroxyguanine (8-OH-Gua) lesions. These nanoparticles were thoroughly characterized with a range of analytical techniques in these published manuscripts [5, 7]. Analytical methods are not described in depth in this conference proceeding as a result of space limitations, but are available in the manuscripts.

RESULTS AND DISCUSSION

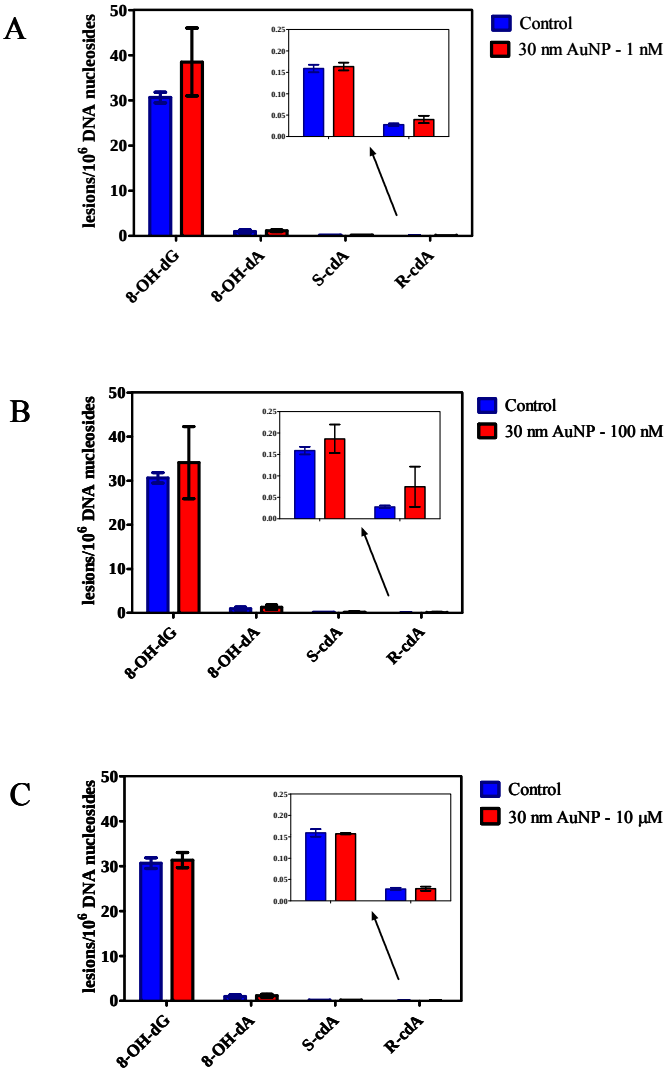

Figure 1: LC/MS/MS DNA damage evaluation of ct-DNA solutions (acellular system) dosed with NIST 30 nm AuNP RMs. (A) Measured lesion levels in the presence of 1 nmol/L AuNP. (B) Measured lesion levels in the presence of 100 nmol/L AuNP. (C) Measured lesion levels in the presence of 10 µmol/L AuNP. Blue: control lesion level. Red: experimental lesion level. The ratio of DNA lesions/10^6 DNA nucleosides represents the mean from three independent samples. The error bars 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 [7].

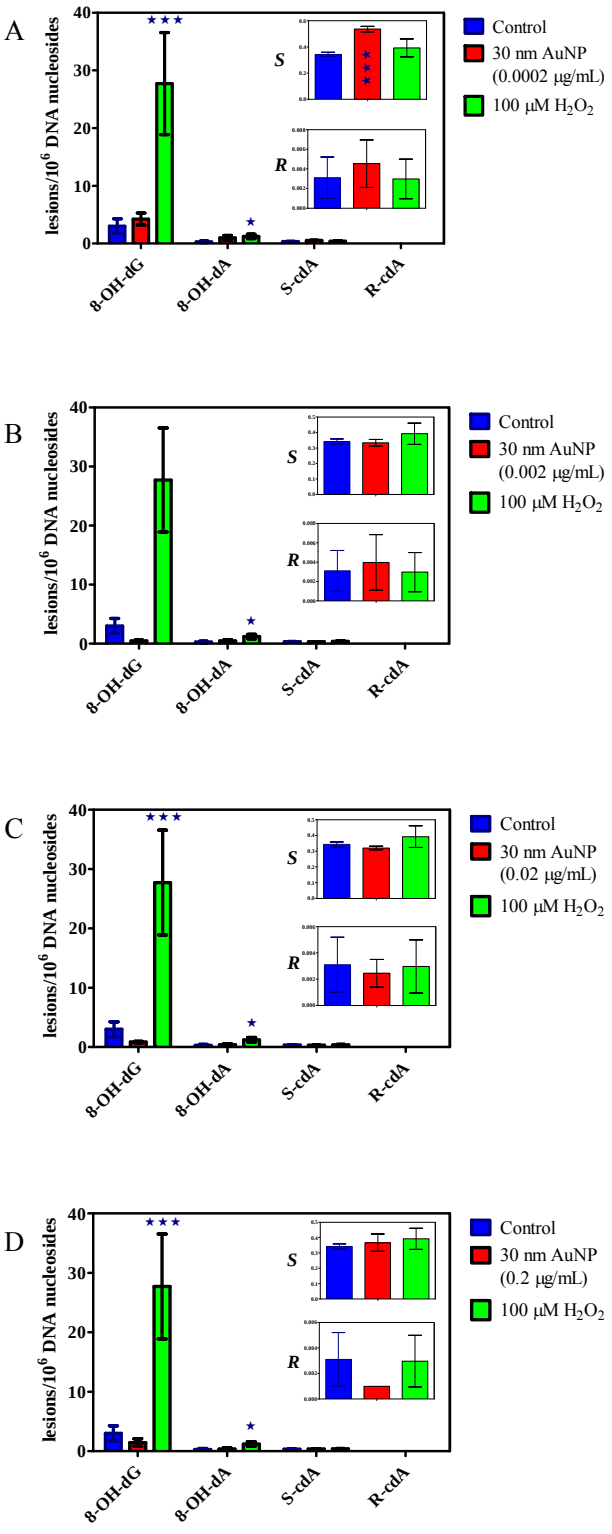

Figure 2: LC/MS/MS DNA damage evaluation of HepG2 cell cultures dosed with NIST 30 nm AuNP RMs. (A) Measured lesion levels in the presence of 1 nmol/L AuNP.

(B) Measured lesion levels in the presence of 10 nmol/L AuNP. (C) Measured lesion levels in the presence of 100 nmol/L AuNP. (D) Measured lesion levels in the presence of 1000 nmol/L AuNP. Blue: control lesion level. Red: experimental lesion level. Green: positive control (H_2O_2) lesion level. The ratio of DNA lesions/10^6 DNA nucleosides represents the mean from three independent samples. The error bars 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 [7].

The primary finding of this study was that NIST RM AuNPs did not cause elevated levels of the lesions studied at this range of AuNP concentrations. While elevated levels of *S*-cdA were observed for the lowest AuNP concentration, these results were not observed at higher concentrations. The results shown in Figures 1 and 2 are for the NIST 30 nm AuNPs, but similar results were obtained for the 10 nm and 60 nm AuNP RMs. Additionally, similar results indicating a lack of genotoxicity were obtained after exposing HepG2 cells for 24 h. The concentration range utilized was chosen to span that which could be used for biomedical applications of AuNPs such as for bioimaging. Thus, these results bode well for the potential application of AuNPs for treatment purposes. Moreover, there is a need for negative nanoparticle controls in nanotoxicology studies. Given the lack of genotoxicity and cytotoxicity observed in this study, these RM AuNPs could potentially fulfill this role given that they have been rigorously characterized and are available to laboratories worldwide with the guarantee of the same particles being delivered across a multiple year period.

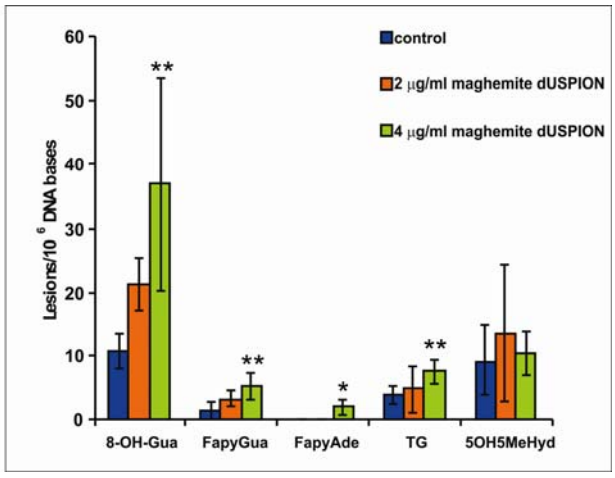

Figure 3: DNA damage in MCL5 cells after exposure to ultrafine superparamagnetic iron oxide nanoparticles (USPION): DNA lesions: 8-OH-Gua, FapyGua, FapyAde, TG and 5-OH-5MeHyd were assessed using GC/MS. *p < 0.05 and **p < 0.01 compared to untreated cells. All data points represent the mean of 5 independent measurements. Uncertainties are standard deviations. Reprinted with permission from [5].

To investigate the impacts of oxidative damage from ultrafine superparamagnetic iron oxide nanoparticles (USPIONs) to biomedically-relevant cells, MCL5 cells were exposed to two concentrations of USPIONs and were found to be non-cytotoxic. This lack of cytotoxicity is important because false positives for DNA damage could be obtained from dead cells if oxidative damage occurs to DNA lysed from the cells [11]. The highest concentration of USPIONs tested had significantly increased DNA lesion levels for four of the five lesions tested. Importantly, the DNA lesion levels were increased in a dose-dependent manner. This finding in combination with those from another study on CuO NP exposed plants indicate that GC/MS procedures previously developed by our group can be used without further modification to measure DNA damage lesion levels caused by nanoparticle exposure [5, 6].

We have many ongoing research projects designed to investigate different aspects of NP-induced oxidative DNA damage to raw DNA, cells, and organisms. One of these projects relates to the potential for carbon nanotubes to cause lesions to AML 12 cells; these cells are being exposed to different NP concentrations and for different durations related to the amount of time it takes for the carbon nanotubes to enter the cells. Another new research direction is utilizing *Caenorhabditis elegans* to assess the extent to which oxidatively-induced lesions are caused by silver nanoparticles or silver ions released by the nanoparticles. Calf thymus is also being used to determine the mechanism by which silver nanoparticles cause genotoxicity. Lastly, several projects are investigating the genotoxicity of a candidate standard reference material nanoscale titanium dioxide (TiO_2). One project examines the potential of dispersed nanoparticles to cause oxidatively-induced DNA lesions to calf thymus DNA under various lighting conditions, while another looks at TiO_2 NP toxicity and uptake into food crops.

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