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Short Communication

Mutagenicity evaluation of metal oxide nanoparticles by the bacterial reverse mutation assay

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ABSTRACT

Nanomaterials have been emerging as a new group of contaminants in the environment. We reported the use of a bacterial reverse mutation assay (Ames assay) to evaluate the mutagenicity of five metal oxide nanoparticles Al_2O_3, Co_3O_4, CuO, TiO_2, and ZnO in this study. Results showed the mutagenicity was negative for four nanoparticles (Al_2O_3, Co_3O_4, TiO_2, and ZnO) up to 1000 μg/plate to all three tested strains without S9 metabolic activation. Using a preincubation procedure and high S9 (9%) activation, TiO_2 and ZnO induced marginal mutagenesis to strain *Escherichia coli* WP2 trp uvrA. CuO displayed low mutagenic potential to *Salmonella typhimurium* TA 97a and TA100 at specific concentrations. However, the colony inhibition effect of CuO was predominant to the strain *E. coli* WP2 trp uvrA. A dose-dependent inhibition of *Escherichia coli* WP2 colony was found under CuO exposure at concentration range of 100–1600 μg/plate. No growth inhibition of tested bacterial strains by Al_2O_3, Co_3O_4, and ZnO was observed at the concentrations used.

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1. Introduction

Nanomaterials have been emerging as a new group of contaminants in the environment. The environmental and human health risks of metal oxide nanomaterials are largely unknown. It is known that physical and chemical properties of nanoparticles are different compared to their bulky counterparts. These properties can be utilized to provide enhanced applications in various fields including imaging, healthcare, cosmetics, and engineering industries. For example, the metal oxide titanium dioxide (TiO_2) is useful in cosmetics due to its UV-blocking properties and can become colorless at its nanosize (~50 nm) (Nasu and Otsubo, 2007; Melquiades et al., 2008). Another metal oxide nanoparticle (NP) CuO also has many commercial applications such as acting as catalyst for the treatment of diesel soot (Braga et al., 2007), as pigments in ceramics, as conductivity enhancement agent in electronics (Hwang et al., 2006), and as ingredients in gun and rocket propellant (Camp and Csanady, 1983). The ZnO NP is also a catalyst and a promising semi-conducting material (Hong et al., 2006; Jung and Choi, 2006). Since significant physical and chemical property alters as the particle size reduced to the nanorange, the biological property of engineered nanoparticle may be different from their larger-sized counterparts. Toxicological studies are needed to develop data sets and novel methodologies necessary for the risk assessment of nanomaterials.

Previous toxicological studies of nanomaterials have been limited and focused only on several "model" nanomaterials including TiO_2, C_{60} and carbon nanotubes. It is reported that TiO_2 induced apoptosis and/or necrosis in human monoblastoid cell U937 (Vamanu et al., 2008), suggesting cytotoxicity. The in vivo evaluation of the toxicity of carbon fullerene using embryonic zebrafish has revealed C_{60} induced both necrotic and apoptotic cellular death throughout the developmental stages of embryo (Usenko et al., 2007). The biochemical and molecular toxicity of TiO_2 and C_{60} has been well studied that indicates oxidative damages, toxicant-metabolism alterations, and DNA damages (Ueng et al., 1997; Sayes et al., 2005; Guo et al., 2007). Although the toxicity of the nanosized TiO_2 has been studied (Warheit et al., 2007), few studies have examined the toxicity of other important metal oxide nanoparticles. We ask if metal oxide nanoparticles with different chemical compositions display similar or instead quite different toxicity properties from the model particle TiO_2.

The bacterial reverse mutation assay (Ames assay) is a classic bioassay to determine the potential genotoxicity of various compounds (Maron and Ames, 1983). Limited studies have used the Ames bioassay to test the potential mutagenicity of TiO_2 using various bacterial strains (Warheit et al., 2007). The aim of this study was to use the Ames assay to investigate and compare the mutagenicity of several metal oxide nanoparticles (TiO_2, Al_2O_3, Co_3O_4, CuO, and ZnO) in order to better define the toxic potential of this group of nanomaterials.

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2. Materials and methods

2.1. Chemicals

Five metal oxide nanoparticles were obtained from Sigma–Aldrich. The particle size of aluminum oxide (Al_2O_3) nanopowder was characterized by the transmission electron microscope (TEM) as <50 nm and has a surface area (SA) of >40 m^2/g determined by the BET method. The cobalt oxide (Co_3O_4) nanopowder was <50 nm with BET surface area of 40–70 m^2/g. The copper oxide (CuO) nanopowder was <50 nm with SA of 29 m^2/g. The titanium dioxide (TiO_2) nanopowder was <100 nm with SA of >14 m^2/g. The zinc oxide (ZnO) nanopowder was <100 nm with SA of 15–25 m^2/g. Calculated amounts of tested nanoparticles, dissolved in double-distilled water (dd water) to give desired quantities in 100 μL water for each plate, were prepared right before plating. Homogenous suspension of nanoparticle in the water solution was obtained by vortex mixing before adding to each agar plate.

2.2. Test bacterial strains

The test strains used were *Salmonella typhimurium* TA97a, TA100, and *Escherichia coli* WP2 trp uvrA (Molecular Toxicology Inc. Annapolis, MD, USA). The S9 fraction from Aroclor 1254 induced rats (Molecular Toxicology Inc. Annapolis, MD, USA) were used for the metabolic activation of tested nanoparticles. Compounds used as positive controls were also from MOLTOX (Molecular Toxicology Inc. Annapolis, MD, USA), and dd water was used as the negative control.

2.3. Mutagenicity assay

A standard plate incorporation procedure was used for the test of without S9 activation. Bacterial strains from disc cultures were grown in LB liquid overnight for 12 h. Overnight bacterial cultures of 100 μL were added to each of the four concentrations of nanoparticles (0, 10, 100, and 1000 μg/plate) dissolved in 100 μL dd water. Then, 2 mL top agar (kept in 45 °C water bath) were immediately added, mixed for 3 s using a vortex mixer, and poured into minimal bottom agar. Plates were incubated for 72 h at 37 °C in a vented incubator. For test of with S9 activation, a modified standard plate incorporation procedure – preincubation assay was used (Maron and Ames, 1983). Briefly, after overnight culture, bacterial strains were added to different concentrations of nanoparticles dissolved in 100 μL dd water, a 0.5 mL S9 mixture (9% S9) was added and incubated for 60 min at 37 °C in a shaken water bath (90 rpm). After the incubation, 2 mL of top agar (kept in 45 °C water bath) were added, mixed for 3 s using a vortex mixer, and poured into the minimal bottom agar. All tested plates (with or without S9 activation) were incubated for 72 h at 37 °C in a vented incubator. After 72 h incubation, his + or trp + revertant colonies were counted within 24 h and recorded in an Excel® spreadsheet. Assays were performed in triplicate. The procedure for CuO test was identical to tests for other nanoparticles, except that six concentrations of CuO were used (0, 100, 200, 400, 800, and 1600 μg/plate).

2.4. Data analysis

Data were processed using the standard statistical software SPSS (SigmaPlot, Version 8.0, and SigmaStat, Version 2.03, SPSS, Chicago, Illinois, USA). Analysis of variance (ANOVA) was used for comparing means of different treatment groups. If there was a significant difference among groups, least significant difference multiple comparisons were conducted to compare the means of each group.

3. Results

Without S9 activation, the mutagenicity of Al_2O_3, Co_3O_4, TiO_2, and ZnO to *S. typhimurium* TA97a, TA100, and *E. coli* WP2 trp uvrA was negative for all tested concentrations (Table 1). With S9 activation, the mutagenicity of Al_2O_3 and Co_3O_4 to all the three bacterial strains was negative at all the tested concentrations, since no significant increase in the number of reverse mutants were found in treatment groups as compared to the dd water controls (Table 2). However, the number of reverse mutant of *S. typhimurium* TA100 was significantly decreased ($p < 0.05$) in all three TiO_2 treated groups (Table 2). This indicates TiO_2 may have low microbial growth inhibition effect. However, such inhibition was not dose-dependent. In contrast, reverse mutants of *E. coli* WP2 trp uvrA were significantly increased at all the three TiO_2 treated groups and reached the highest at the concentration of 1000 μg/plate (Table 2). The mutagenicity index (MI, defined as the average number of reverse mutants of treated group/the average number of reverse mutants of negative control group) of TiO_2 to *E. coli* WP2 strain were 1.4, 1.3, and 1.8 for 10, 100, and 1000 μg/plate, respectively. In addition, ZnO also induced significantly more reversed colonies of *E. coli* WP2 at 1000 μg/plate with MI of 1.4 (Table 2). This suggests low but notable mutagenic potential of TiO_2 and ZnO to this strain of bacteria.

At concentrations up to 1000 μg/plate, CuO nanoparticles were not mutagenic to *S. typhimurium* TA97a without S9 activation (Fig. 1a). However with S9 activation, CuO seemed to inhibit TA97a and was significant at 100 μg/plate treatment (Fig. 1b). Surprisingly, at 1000 μg/plate treatment, the reverse mutants were significantly increased compared to control with a mutagenicity index of 1.3 (Fig. 1b). This suggests CuO may have mixed effects (both cytotoxic and mutagenic) to this strain. We further tested effects of CuO on *S. typhimurium* TA100 and *E. coli* WP2 trp uvrA at concentration range of 100–1600 μg/plate with two times increase in each dose. Without S9 activation, CuO induced significantly more reverse mutants of *S. typhimurium* TA 100 at all five treated concentrations (Fig. 2). The MIs for 100, 200, 400, 800, and 1600 μg/plate treatments were 1.2, 1.2, 1.2, 1.3, and 1.3, respectively. This suggests marginal mutagenic effects of CuO nanoparticle to *S. typhimurium* TA100. In contrast, CuO treatments significantly inhibited the growth of *E. coli* WP2 trp uvrA. This inhi-

Table 1
The number of reverse mutants of *S. typhimurium* TA97a, TA100, and *E. coli* WP2 by the nanoparticles Al_2O_3, Co_3O_4, TiO_2, and ZnO at various doses without S9 activation.[a]

Compound	Dose (μg/plate)	Number of reverse mutants/plate (mean ± SD)		
		TA97a	TA100	WP2
Al_2O_3	0	205 ± 13	322 ± 14	237 ± 55
	10	189 ± 1	303 ± 7	276 ± 9
	100	240 ± 8	356 ± 88	278 ± 17
	1000	217 ± 25	324 ± 6	307 ± 1
Co_3O_4	0	205 ± 13	322 ± 14	237 ± 55
	10	220 ± 18	379 ± 36	314 ± 35
	100	201 ± 43	340 ± 10	309 ± 37
	1000	214 ± 9	355 ± 3	296 ± 25
TiO_2	0	205 ± 13	322 ± 14	237 ± 55
	10	223 ± 10	352 ± 19	185 ± 1
	100	203 ± 13	331 ± 1	188 ± 4
	1000	189 ± 5	270 ± 54	188 ± 15
ZnO	0	205 ± 13	322 ± 14	237 ± 55
	10	201 ± 16	300 ± 34	285 ± 33
	100	204 ± 18	309 ± 23	253 ± 33
	1000	187 ± 13	320 ± 13	196 ± 31

[a] Positive controls: 2-amine-fluorene for TA97a, 605 ± 22 colonies; sodium azide for TA100, 686 ± 18 colonies; N-Ethyl-N-nitro-nitrosoguanidine for WP2, 653 ± 30 colonies.