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A review of toxicity studies on graphene-based nanomaterials in laboratory animals

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ABSTRACT

We summarized the findings of toxicity studies on graphene-based nanomaterials (GNMs) in laboratory mammals. The inhalation of graphene (GP) and graphene oxide (GO) induced only minimal pulmonary toxicity. Bolus airway exposure to GP and GO caused acute and subacute pulmonary inflammation. Large-sized GO (L-GO) was more toxic than small-sized GO (S-GO). Intratracheally administered GP passed through the air-blood barrier into the blood and intravenous GO distributed mainly in the lungs, liver, and spleen. S-GO and L-GO mainly accumulated in the liver and lungs, respectively. Limited information showed the potential behavioral, reproductive, and developmental toxicity and genotoxicity of GNMs. There are indications that oxidative stress and inflammation may be involved in the toxicity of GNMs. The surface reactivity, size, and dispersion status of GNMs play an important role in the induction of toxicity and biodistribution of GNMs. Although this review paper provides initial information on the potential toxicity of GNMs, data are still very limited, especially when taking into account the many different types of GNMs and their potential modifications. To fill the data gap, further studies should be performed using laboratory mammals exposed using the route and dose anticipated for human exposure scenarios.

1. Introduction

Humans are continually exposed to naturally occurring nanoparticles. In addition, the rapidly developing field of nanotechnology, which is creating materials with size-dependent properties. Actually it already became a source of exposure, considering the growing number of engineered nanomaterial (ENM) occupational exposure assessment studies. The ENMs have

Abbreviations: Ach, acetylcholine; ADD, aerodynamic diameter; AgD, agglomerate density; AgS, Agglomerate size; ApD, apparent density; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BALF, bronchoalveolar lavage fluid; BBS, balanced buffer solution; BSA(-), bovine serum albumin(-coated); BSS, balanced salty solution; BUN, blood urea nitrogen; CDD, cumulative deposition dose; CDAgD, calculated deposition using agglomerate density; CDApD, calculated deposition using apparent density; CINC, cytokine-induced neutrophil chemoattractant; COOH-, carboxylated; CRE, creatinine; CSAg/P, cross section of agglomerates/particles; D, diameter; DEX-, dextran functionalized; ED, exposure dose; ENM, engineered nanomaterial; EHD, equivalent hydrodynamic diameter; F_{χ}-, F functionalized; GD, gestational day; GGT, γ-glutamyl transpeptidase; GLP, good laboratory practice; GM-CSF, granulocyte macrophage colony-stimulating factor; GNMs, graphene-based nanomaterials; GO, graphene oxide; GP, graphene; GQD, graphene quantum dot; GSD, geometric standard deviation; GSH, glutathione; GSH-PX, glutathione peroxidase; HCT, hematocrit; HDD, hydrodynamic diameter; HDS, hydrodynamic size; HGB, hemoglobin; ICP-MS, inductively-coupled plasma mass spectrometry; IL, interleukin; L-, large-sized; LA-PEG-, lactobinoic acid-polyethylene glycol functionalized; LD, lateral dimension; LDH, lactate dehydrogenase; MCHC, mean corpuscular hemoglobin concentration; MCP, monocyte chemotactic protein; MDA, malondialdehyde; MIP, macrophage inflammatory protein; MMAD, mass median aerodynamic diameter; MMP, matrix metallopeptidase; MNPCE, micronuleated polychromatic erythrocyte; MWCNT, multi-walled carbon nanotube; N_2-, acylated; NF, nuclear factor; NH_2-, aminated; NM, nanomaterial; O_2-, O^+ fuctionalized; OH-, hydroxyl; PAA-, poly(acrylic acid)-functionalized; PAM-, poly(acrylamide)-fuctionalized; PBS, phosphate-buffered saline; PCE/NCE, ratio of polychromatic erythrocytes to normochromatic erythrocytes; PEI-, polyethylenimine functionalized; PEG-, polyethylene glycol functionalized; PF-, PF108-dispersed; PL, peritoneal lavage; PLT, platelet; PMNs, polymorphonuclear leucocytes; PND, postnatal day; PSS-, sodium 4-styrenesulfonate functionalized; RBC, red blood cell; RES, reticuloendothelial system; rGO, reduced graphene oxide; ROS, reactive oxygen species; S, size; S-, small-sized; SA, surface area; SA-BET, surface area by Brunauer-Emmett-Teller method; SWCNT, single-walled carbon nanotube; Sh, size in H_2O; SOD, superoxide dismutase; T, thickness; TAT, thrombin-antithrombin; TEM, transmission electron microscope; TGF, tumor growth factor; TLR, toll-like receptor; TNF, tumor necrosis factor; TUNEL, TdT-mediated dUTP nick end labeling; WBC, white blood cell; ZP, zeta potential.

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an increased surface area:mass ratio, greatly enhancing their chemical/catalytic reactivity compared with normal-sized forms of the same substance. Human exposure can occur at any stage during the life cycle, including the synthesis, manufacture, use, and disposal, of ENMs (Jiménez et al., 2014). The introduction of these novel materials into the work environment and consumer products necessitates safety evaluations as well as a clearer understanding of any potential impact on human health. Nanomaterials (NMs) have the ability to pass through biological membranes (Rothen-Rutishauser et al., 2007); therefore, they may be able to affect the physiology of any cell in the body. The potential of chemicals to enter biological systems is a matter of great concern to the general public. At present, however, information on the toxicological effects of ENMs is very limited.

Graphene (GP) is the new allotrope of carbon and is defined as a single layer of monocrystalline graphite with sp^2-hybridized carbon atoms tightly packed in a two-dimensional honeycomb lattice, resulting in a large surface area on both sides of the planar axis (Duch et al., 2011; Bianco, 2013; Seabra et al., 2014). GP-based NMs (GNMs) include single-layer GP, few-layer GP (2–10 layers), GP oxide (GO) (normally a single layer), reduced GO (rGO) (normally a single layer), GP nanosheets, GP ribbons, and GP quantum dots (GQDs) (Sanchez et al., 2012; Bianco, 2013). GNMs have unique electronic and mechanical properties, specific magnetism, excellent mobility of charge carriers, high thermal conductivity, a large surface area, excellent conductivity, outstanding mechanical strength, and extraordinary electrocatalytic activity (Jastrzębska et al., 2012), as well as potential biocompatibility, which make them attractive candidates for biomedical applications including electrochemical devices, energy storage, absorption of enzymes, cell imaging, drug delivery, and biosensors (Xu et al., 2013).

GNMs are newly developed substances; single GP layers were first isolated by the mechanical exfoliation of highly oriented pyrolytic graphite (Novoselov et al., 2004). To date, several review papers regarding the toxicity of GNMs have been published (Jastrzębska et al., 2012; Sanchez et al., 2012; Bianco, 2013; Xu et al., 2013; Guo and Mei, 2014; Nezakati et al., 2014; Seabra et al., 2014; Lalwani et al., 2016). However, these review papers provided insufficient information about in vivo toxicity studies using mammalian species because toxicity studies of GNMs have been rarely performed until very recently and the amount of literature on this topic has only just started to increase. It is very important, from time to time, to take a break and summarize and re-assess the accumulating data, because ongoing and future research along with new findings are the only productive ways to assess risks and potential hazards associated with the use of new materials (Shvedova et al., 2016). In this review, we focused on the significant toxic effects of GNMs, published as openly available scientific literature, in laboratory mammals because the use of mammalian species is essential for the realistic testing of toxicity. The objective of this review is to evaluate what is currently known about the toxicity of GNMs and to understand the potential risk posed by them.

2. Data from animal research

2.1. Pulmonary toxicity

2.1.1. Pulmonary toxicity in rats exposed by inhalation

Four nose-only inhalation studies are available on the pulmonary toxicity of GP and GO (Table 1).

Rats inhaled GP at 0.54, 3.05, or 10.1 mg/m^3 for consecutive 5 days, 6 h/day (Ma-Hock et al., 2013). Test atmospheres with GP were produced with swinging bed dust generator. Generation dusts were mixed with compressed air in a glass tube, diluted with conditioned air and passed via a cyclone into the inhalation system. The aerodynamic diameters of the agglomerates were mostly

Table 1
Pulmonary toxicity of graphene (GP) and graphene oxide (GO) given to rats by nose-only inhalation.

Test materials	Test atmospheres	Animals (No. of rats/group)	Measured mass concentrations	Observation after exposure	Findings	Reference
GP (D: < 10,000 nm, C: 84.1, O: 8.8, S: 5.4, Na: 0.6, Si: 0.4, Cl: 0.6%, flake)	(At highest conc.) MMAD: ≤ 0.4 μm, ApD: 0.04 g/mL, CDApD: 0.26 mg/lung, AgD: 0.29 g/mL, CDAgD: 0.30 mg/lung	Male Wistar rats (8)	0.54, 3.05, 10.1 mg/m^3 (6 h/day, 5 days)	3, 24 days	↑ PMNs, CINC-1/IL-8, and MCP-1 at ≥ 3.05 mg and lymphocytes at 10.1 mg on days 3 and 24, eosinophils, and osteopontin at 3.05 and/or 10.1 mg on day 3 and/or 24, and CINC-1 and osteopontin at 0.54 mg in BALF; IL-1α at ≥3.05 mg on day 3 in lung tissue; particle-laden macrophages or aggregates of macrophages and a few microgranulomas in the lung at ≥3.05 mg.	Ma-Hock et al. (2013)
GP (T: 8 nm, LD: ~550 nm, SA: 100 m^2/g, C: 76.8, O: 10.36, Na: 10.49, P: 2.36 in weight %, flake, Graphene Supermarket, USA)	S: 10–130 nm, MMAD: 567 nm, GSD: 2.4, CDD: 0.018 mg/lung (low conc)/0.102 mg/lung (high conc.)	Male SD rats (15)	0.68, 3.86 mg/m^3 (6 h/day, 5 days)	1-28 days	↓ Body weight during 2nd week at ≥ 0.68 mg; WBCs and lymphocytes on day 3 at 3.86 mg in blood. ↑ Neutrophils, lymphocytes, and monocytes on day 1, MCHC on day 28 in blood; a slight thickening of the alveolar wall at 3.86 mg on days 1–7; alveolar macrophages ingested GP at ≥ 0.68 mg on days 1–28. No clinical changes.	Shin et al. (2015)
GP (LD: < 2 μm, SA: 750 m^2/g, density: 0.2 g/mL, 20–30 layers, Cabot Corp., USA)	T: 0.35–0.38 nm, MMAD: 123 nm, GSD: 3.63, CDD: 0.012 mg/lung (low conc)/0.05 mg/lung (moderate conc.)/0.198 mg/lung (high conc.)	Male SD rats (15)	0.12, 0.47, 1.88 mg/m^3 (4 weeks, 6 h/day, 5 days/week)	1, 28, 90 days	No dose-dependent effects on body weight, organ weight, BALF inflammatory parameters, or blood chemistry. No genotoxicity in comet assay using inhaled lung tissues.	Kim et al. (2016)
GO (EHD: 150–250 nm, C: 56.8, O: 20.2, Na: 8.3, Cl: 3.4, K: 11.3 in weight %)	S: 10–120 nm, 50.6 nm in D 6.45×10^{10} nm^2/cm^3 in SA of low conc., 72.9 nm in D and 2.72×10^{11} nm^2/cm^3 in SA of high conc.	Male SD rats (12)	0.46, 3.76 mg/m^3 (single 6-h exposure)	1, 7, 14 days	↑ MMP-9 on day 1 and IL-18 and TGF-β1 on day 7 in BALF and alveolar macrophage ingested GO at 3.67 mg. No effects on other inflammatory parameters in BALF, body weight, food intake, or weight or gross changes of organs.	Han et al. (2015)