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Developmental toxicity of engineered nanomaterials in rodents

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ABSTRACT

We summarized significant effects reported in the literature on the developmental toxicity of engineered nanomaterials (ENMs) in rodents. The developmental toxicity of ENMs included not only structural abnormalities, but also death, growth retardation, and behavioral and functional abnormalities. Most studies were performed on mice using an injection route of exposure. Teratogenic effects were indicated when multi-walled carbon nanotubes (MWCNTs), single-walled carbon nanotubes (SWCNTs), and TiO_2-nanoparticles were administered to mice during early gestation. Reactive oxygen species levels were increased in placentas and malformed fetuses and their placentas after prenatal exposure to MWCNTs and SWCNTs, respectively. The pre- and postnatal mortalities and growth retardation in offspring increased after prenatal exposure to ENMs. Histopathological and functional abnormalities were also induced in placentas after prenatal exposure to ENMs. Maternal exposure to ENMs induced behavioral alterations, histopathological and biochemical changes in the central nervous system, increased susceptibility to allergy, transplacental genotoxicity, and vascular, immunological, and reproductive effects in offspring. The size- and developmental stage-dependent placental transfer of ENMs was noted after maternal exposure. Silver accumulated in the visceral yolk sac after being injected with Ag-NPs during early gestation. Although currently available data has provided initial information on the potential developmental toxicity of ENMs, that on the developmental toxicity of ENMs is still very limited. Further studies using well-characterized ENMs, state-of the-art study protocols, and appropriate routes of exposure are required in order to clarify these developmental effects and provide information suitable for risk assessments of ENMs.

1. Introduction

Donaldson et al. in 2004 indicated that the discipline of nanotoxicology needed to be developed further in order to address new potential threats associated with the widespread use of new nanomaterials (NMs) and to address the growth of a safe and sustainable nanotechnology industry. The rapidly developing field of nanotechnology creates materials with size-dependent properties and is likely to become another source of exposure to nanosized materials. These NMs have an increased surface area:mass ratio, which greatly enhances their chemical/catalytic reactivity over that of bulk-sized forms of the same substance. 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.

Nanoparticles (NPs) 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 with regards to developmental toxicity because living matter is more susceptible to adverse insults during early development than at any other time in the life cycle. Developmental toxicity refers to the adverse effects of chemical compounds or physical agents on developmental processes until their completion. Developmental toxicity includes not only structural abnormalities (malformation), but also death, growth retardation, and behavioral and functional abnormalities. Toxic outcomes and susceptibility varies with the developmental stage at the time of exposure to toxic insults. The timing of exposure is a critical consideration in developmental toxicity studies because many crucial developmental events occur in a very narrow time window, especially in rodents, which have a very short gestation period (Kimmel and Price, 1990).

Developmental toxicity is increasingly becoming recognized as an important part of overall toxicology. However, the developmental toxicity of NMs has not been much studied until very recently. We previously reviewed the reproductive and developmental toxicities of manufactured NMs (Ema et al., 2010) and carbon-based NMs (Ema et al., 2015), and Hougaard et al. (2015) exhaustively reviewed the developmental toxicity of inhaled NPs. However, a recent review is unavailable for the developmental toxicity of whole engineered NMs (ENMs) regardless of administration routes in experimental animals. In this review, we highlighted recent findings and briefly summarized significant effects reported in the literature on the developmental toxicity of ENMs in mammalian species because the mammalian maternal–placental–embryonic/fetal relationship is essential for the realistic testing of developmental toxicity.

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Table 1
Developmental effects reported in the literature on toxicity studies on engineered nanomaterials (ENMs) in rodents.

Effects on morphological development, growth, and survival	Effects on behavior/CNS	Immunological, cardiovascular, and genotoxic effects	Reproductive effects	Placental transfer
External and skeletal malformations Visceral variations Delayed development Abortion Pre- and postnatal mortality Decreased body weight of fetuses and neonates Decreased fetal length Increased ROS in malformed fetuses and placentas Placental abnormality (decreased size, vascular damage, reduced blood flow)	Increased time spent in the closed arms of the elevated plus-maze and floating in forced swimming test Change in open-field behavior (habituation, time spent, and frequency of visits in the central zone) Impaired cognitive behavior in the Morris water maze and passive avoidance test Change in passages in the light–dark box Enhancement in prepulse inhibition Degenerated PAS-positive granules and astrocytic endfeet Increased GFAP levels in the astrocytic endfeet Increased number of cells positive for caspase-3 in the olfactory bulb Increased levels of dopamine and metabolites in the prefrontal cortex and neostriatum Impaired synaptic plasticity and cell proliferation in the hippocampus Decreased levels of GAP-43 mRNA in the hippocampus	Increased susceptibility to allergy Changed phenotypes of thymocytes and splenocytes Upregulated gene expression related to T cell survival and induction of peripheral tolerance Abolishment of endothelium-dependent reactivity, impairment of endothelium-dependent dilation and active mechanotransduction in coronary and uterine arteries, and reduction in mitochondrial respiration DNA damage in the fetal liver and placenta DNA strand breaks in the fetal liver Increased frequency of MNPCEs in the fetal liver and peripheral blood	Delayed delivery of the first litter Decreased DSP Decreased DSP in F2 males Decreased sperm motility Histopathological abnormality in testes (vacuolation and deformation of the seminiferous tubules, tubule lumens with a few mature sperm, and deformation of the spiral artery)	MWCNTs in the fetal liver and placenta after iv injection on GD 15.5 SWCNTs in the placenta and yolk sac after iv injection on GD 14.5 C_{60} in the placentas and fetal tissues, and milk and pup tissues after iv injection on GD 15 and PND 8, respectively SNP70 and R-TiO_2-NP_s in the placenta, fetal liver, and fetal brain after iv injection on GD 16 A-TiO_2-NPs in Leydig and Sertoli cells, spermatids, olfactory bulb, and the cerebral cortex after sc injection on GDs 3, 7, 10, and 14 Accumulation of Ag in the visceral yolk sac and embryos after iv injection on GDs 7–9, Ag-NPs in fetuses and milk/neonates after gastric intubation on GD 20 and PNDs 14–16, respectively Au-NPs in embryos at higher levels after iv injection before GD 11.5 and decreased levels thereafter in embryos, Au-NPs in fetuses and enhanced placental transfer by intrauterine inflammation Accumulation of Cd in pre- and postnatal offspring after iv injection of CdTe/CdS QDs during late gestation

2. Developmental effects reported in the literature

The developmental toxicity of ENMs has been studied in mice and rats, but mainly in mice. Table 1 shows significant effects on development reported by toxicity studies performed on rodents.

3. Effects on morphological development, growth, and survival

Multi-walled carbon nanotubes (MWCNTs) increased the maternal incidence of fetuses with external and skeletal malformations, including defects in the tail, limb, ribs, and vertebrae, after a single intratracheal instillation at 2 mg/kg and higher or an intraperitoneal injection at 4 mg/kg and higher to mice on gestational day (GD) 9 (Fujitani et al., 2012). MWCNTs also caused low fetal body weight and survival rate.

Intravenous injection of oxide (o)-MWCNTs induced abortion in mice at 20 mg/kg from GD 7 until abortion or parturition (Qi et al., 2014). Reactive oxygen species (ROS) levels were increased in the placenta, but not in maternal plasma. Histopathology revealed that o-MWCNTs decreased the number of blood vessels and narrowed blood vessels in the placentas. Fetal growth retardation and death appeared to be associated with placental dysfunction.

Amine-functionalized polyethylene glycol (AF-PEG)-MWCNTs were intravenously injected at 2 mg/kg in pregnant $p53^{+/-}$ mice on GDs 10.5, 12.5, and 15.5 (Huang et al., 2014). MWCNTs with a diameter of ~50 nm (MWCNT-50), but not MWCNTs with smaller diameters (<8 and 20–30 nm), increased the incidence of brain defects. MWCNT-50 and -20 decreased fetal body weight and MWCNT-50 decreased postnatal survival of offspring. Brain defects were induced in p53 knockout fetuses in a p53 status-dependent manner. MWCNT-50 at 5 mg/kg on GD 10.5 caused brain defects in half of the $p53^{-/-}$ fetuses tested. The co-injection of an antioxidant markedly decreased the number of $p53^{-/-}$ fetuses with brain defects. These findings suggest that p53, a tumor suppressor gene, is important as a teratogenic suppressor gene and oxidative stress and diameter are determining factors for the teratogenicity of MWCNTs. However, none of the teratogenic effects of AF-PEG-single walled carbon nanotubes (SWCNTs) were detected in $p53^{+/-}$ mice intravenously injected on GDs 10.5, 12.5, and 15.5.

The incidence of mouse dams with malformed fetuses increased when 3 μg/mouse of pristine (p)-SWCNTs, 30 μg/mouse of o-SWCNTs, and 0.3 μg/mouse and higher of ultra oxide (u)-SWCNTs were administered intravenously on GD 5.5 (Pietroiusti et al., 2011). Deformities in the abdominal wall and head and limb hypoplasia were observed. The placentas of malformed fetuses were small and vascular-damaged in the labyrinth layer. Following an injection of uo-SWCNTs, the levels of ROS were higher in malformed fetuses and their placentas. The incidence of abortion was increased in mice injected with p-, o-, and uo- SWCNTs at 30 μg/mouse on GD 5.5. These findings suggest that oxidative stress resulting from the induction of ROS contributes to developmental toxicity and that functionalization markedly changes toxicity.

The occasional teratogenicity of AF-PEG-SWCNTs was observed after an intravenous injection of 30 μg/mouse on GD 5.5 or of 10 μg/mouse on GDs 5.5, 8.5, and 11.5 (Campagnolo et al., 2013). Delayed development and abnormalities in the head and paws were observed. Fetal anomalies occurred concomitantly with placental abnormalities.

Oral gavage of SWCNTs functionalized with hydroxyl (–OH) group (FOH)-SWCNTs has been suggested to be teratogenic in mice (Philbrook et al., 2011a). The incidence of resorptions and fetuses with gross defects or skeletal abnormalities was increased after the administration of 10 mg/kg, but not 100 mg/kg, on GD 9.

Reduced graphene oxide was intravenously injected at 6.25–25 mg/kg during late gestation in mice (Xu et al., 2015). Some dams died and surviving dams aborted their fetuses.

Oral gavage of rutile (R)-TiO_2-NPs at 100 and 1000 mg/kg to mice on GD 9 increased the number of fetal malformations, including exencephaly, open eyelids, and leg and tail defects (Philbrook et al.,