Reproductive Toxicology 41 (2013) 86–97

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Effects of lung exposure to carbon nanotubes on female fertility and pregnancy. A study in mice

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ARTICLE INFO

Article history:
Received 1 March 2013
Received in revised form 15 May 2013
Accepted 17 May 2013
Available online 25 May 2013

Keywords:
Multiwalled carbon nanotubes
Pregnancy
Developmental toxicity
Reproductive toxicity
Nanotoxicology
Lung and liver pathology

ABSTRACT

We studied the effects of preconceptional exposure to multiwalled carbon nanotubes (MWCNTs): mature, female C57BL/6J mice were intratracheally instilled with 67 μg NM-400 MWCNT, and the following day co-housed with mature males, in breeding pairs. Time to delivery of the first litter, litter parameters, maternal inflammation and histopathology of lung and liver were recorded. In male offspring, locomotor activity, startle response, and daily sperm production (DSP) were assessed. In the dams, lung and liver bore evidence of MWCNT exposure when assessed 6 weeks and 4 months after exposure. A short delay in the delivery of the first litter was observed in exposed females. Litter parameters, behavior and DSP were similar in control and exposed groups. In conclusion, instillation of a single dose of MWCNT induced long lasting pathological changes in dam lung and liver. Theoretically, lung inflammation due to particle exposure could interfere with female reproductive parameters. Whether the observed lag in delivery of a first litter was in fact caused by exposure to MWCNT should be addressed in a study designed specifically to elucidate effects on the early processes involved in establishment of pregnancy. Exposure was not associated with changes in the assessed gestational or offspring parameters.

Abbreviations: ANCOVA, analysis of covariance; ANOVA, analysis of variance; ASR, acoustic startle reaction; AVG, average of tube movements for 100 ms following onset of startle stimulus; BAL, bronchoalveolar lavage; COX, cyclooxygenase; CNT, carbon nanotube; dB(A), decibel, A-weighted; DLS, dynamic laser scattering; DSP, daily sperm production; CNT, carbon nanotube; CRP, C-reactive protein; ENP, engineered nanoparticles; EPA, Environmental Protection Agency (USA); EU, endotoxin units; ICP-OES, inductively coupled plasma-optical emission spectrometry; HE, hematoxylin and eosin; IL, interleukin; GD, gestation day; MWCNT, multiwalled carbon nanotube; NIOSH, National Institute of Occupational Safety and Health (USA); PND, postnatal day; PPI, prepulse inhibition; R_i, optical refractive index; R_a, optical absorption index; ROS, reactive oxygen species; SAP, serum amyloid protein; SD, standard deviation; SEM, standard error of the mean; SWCNT, single walled carbon nanotube; TEM, transmission electron microscopy; TGA, thermogravimetric analysis; TNF, tumor necrosis factor; XRD, X-ray diffraction.

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0890-6238
http://dx.doi.org/10.1016/j.reprotox.2013.05.006

1. Introduction

Nanomaterial research and development progress at a high pace, and engineered nanoparticles are continuously introduced to the market [1]. This highlights the need for toxicological assessment, as the risk of exposure of workers and consumers increases. Reproductive and developmental toxicity is integrated into, e.g., the US EPA's nanomaterials research strategy [2] and investigation hereof is recommended by the Reproductive Health Research Team under NIOSH' National Occupational Research Agenda [3]. As yet, this area of nanotoxicology has received little attention [4,5].

Carbon nanotubes (CNTs) have attracted huge industrial interest due to their unique properties [6]. CNTs are high aspect ratio nanomaterials. Although CNTs are thinner, some types possibly have asbestos-like properties [7,8]. The airways are considered the most critical route of worker and consumer exposure [9]. As fibers, CNTs can be too long to be engulfed and removed by macrophages with very long retention times in the lungs as a consequence. A half-life approaching one year was estimated in one study of inhaled MWCNTs in rats [10]. Pulmonary exposure to CNTs induces sustained pulmonary inflammation characterized by neutrophil influx and cytokine production, fibrosis, etc. [10–12].

Following pulmonary exposure to nanoparticles, inflammatory mediators are released from lung cells into circulation, potentially leading to systematic inflammation [13]. Thus, serum levels of the acute phase proteins CRP, SAP and heptoglobin and the cytokine MIP-2 were increased 24 h after aspiration of 40 μg/animal MWCNT and SWCNT [14,15]. Other means of systemically propagating the lung inflammatory response have recently been rendered possible. In a small controlled exposure study, healthy adult human volunteers were exposed to filtered air or diluted diesel exhaust, and RNA was isolated from peripheral blood mononuclear cells and analyzed by microarray analysis. Findings indicate transcriptional changes in inflammatory genes [16]. If activated mononuclear cells travel from the lungs into the circulation, it might have important implications for female reproductive function. During, e.g., the secretory phase of the menstrual cycle, the uterine endometrium undergoes a dramatic invasion of monocytes. These differentiate into tissue macrophages to create the inflammatory and degradative environment of menstruation. In case of implantation, menstruation is prevented, possibly due to a process of leukocyte apoptosis occurring in the vicinity of the implantation site, creating a local anti-inflammatory and implantation friendly environment [17]. Recruitment of cells primed toward an inflammatory phenotype might be hypothesized to impact adversely on reproductive processes as these generally display high sensitivity to changes in redox status [18].

Studies specifically investigating the inflammatory response in the uterus, ovaries and the fetus following lung exposure to CNTs are to our knowledge not available, but some data are available for other types of nanosized particles. Gavage of titanium dioxide nanoparticles (10 mg/kg for 90 consecutive days) thus increased ovarian ROS levels and changed the expression of several ovarian genes, but in the presence of increased levels of titanium in the ovaries [19]. Maternal airway exposure to diesel exhaust in gestation has been found to increase mRNA levels of inflammatory cytokines in the placenta of, e.g., TNF-α, IL-6, and keratinocyte-derived cytokines [20,21]. These results indicate that particulate exposures are capable of inducing inflammatory responses and possibly conditions of oxidative stress in female reproductive organs, and inflammation has been linked to adverse effects on the course of gestation and fetal development [22–25].

Inflammation may also interfere with ovulation. A series of studies in sheep elegantly show how inflammation, as a result of exposure to endotoxin, interfered with several steps in the preovulatory chain of endocrine events and thereby with ovulation. Endotoxin inhibited among others the pulsatile secretion of luteinizing hormone secretion, which provides an essential stimulus for the increase in secretion of oestradiol from the ovarian follicle preceding ovulation (overview provided in [26]). This disruption probably takes place within a sensitive time window of a few hours [27]. Interestingly, in the rat specifically IL-1β and TNF-α, but not IL-6, inhibited the secretion of luteinizing hormone and gonadotropin releasing hormone at the level of the hypothalamus. IL-1β and TNF-α may therefore represent the major proinflammatory cytokines mediating suppression of the reproductive axis in inflammation [28]. Endotoxin exposure potently elevates TNF-α [28,29], and airway exposure of rodents to several types particles has also been associated with increased levels of TNF-α in lung fluid [30]. Direct effects of ENPs on central regulation of sex hormones that might indirectly interfere with reproductive processes have not been specifically investigated so far. However, polyelectrolyte multilayer coated gold nanoparticles (a potential nano-drug) have been reported to accumulate among others in the hypothalamus. Interaction of ENPs with the hypothalamic–pituitary–gonadal axis might therefore be hypothesized [4,31].

All together these data suggest that particle induced lung inflammation may interfere with female reproductive processes. Furthermore, a recent study report overt fetotoxicity after intravenous injection of different types of MWCNTs at very low dose levels [32]. The present study was initiated to investigate whether airway exposure to MWCNTs agglomerates, a potent inducer of airway inflammation, would interfere with reproductive and developmental measures. Due to the potential involvement of maternal lung inflammation in developmental toxicity, assessment of maternal inflammatory response and histopathology of lung and liver were included.

2. Materials and methods

2.1. Background for choice of study design

In a gestational exposure study (Table 1), time mated mice ($n = 22$ per group, C57BL/6JBomTac, Taconic Europe, Ejby, Denmark) were exposed by intratracheal instillation to 67 μg four times during gestation of either NRCWE-006 (XNRI MWNT-7, Mitsui, Japan [33]) or NM-400 from the OECD Working Party on Manufactured Nanomaterials sponsorship programme [25,34] (on gestational days 8, 11, 15 and 18, total dose 268 μg/animal). Controls received the 40 μL of vehicle (Nanopure water with 2% mouse serum, a pooled preparation obtained from a normal mouse population (M5905, Sigma–Aldrich)). When lung inflammation was evaluated by assessment of cellular composition in BAL fluid, neutrophilia was obvious in the exposed females as long as 25 days after the last day of exposure. However, also eosinophilia was observed in BAL fluid in all groups at PND 2. Subsequent analysis of the mouse serum revealed a high content of endotoxin in the commercial mouse serum used for preparation of instillation vehicle. Approximately three quarters of the controls delivered a litter, compared to only half in the MWCNT groups (Table 2). No differences were observed for number of implantations and litter size, so MWCNT exposure had potentially affected early implantation in some females. Notwithstanding the endotoxin contamination, this skewing in delivering females between control and exposed females prompted us to initiate a follow-up study. The potential effects had taken place early in gestation and MWCNT-induced inflammation was furthermore hypothesized to interfere with the processes leading to ovulation. Since inflammation due to lung exposure to MWCNTs lasts for some time ([15] and present study), the mature females were exposed prior to cohabitation with a mature male, and time to delivery of a first litter was chosen as the main endpoint, as this would detect both effects on ovulation and establishment of pregnancy. NM-400 was chosen as the test material in the follow-up study, as differential cell counts of BAL fluid in the gestational exposure study indicated lung inflammation persisted longest for this MWCNT. The overall designs of the gestational and the preconception exposure studies are shown in Table 1.

2.2. Animals

Naïve mice, 60 females and 60 males (C57BL/6JBomTac, Taconic Europe, Ejby, Denmark), were supplied at 7 and 9 weeks of age, respectively. Upon arrival, the animals were distributed to cages, each holding five animals and housed in the same animal room. After one week, the females were weighed and assigned to two groups each of 30 animals, with similar weight distributions. A handful of soiled bedding from male cages was regularly added to female cages, to keep females cycling.