OBSTETRICS

Maternal engineered nanomaterial exposure and fetal microvascular function: does the Barker hypothesis apply?

Phoebe A. Stapleton, PhD; Valerie C. Minarchick, BS; Jinghai Yi, PhD; Kevin Engels, BS; Carroll R. McBride, BS; Timothy R. Nurkiewicz, PhD

OBJECTIVE: The continued development and use of engineered nanomaterials (ENM) has given rise to concerns over the potential for human health effects. Although the understanding of cardiovascular ENM toxicity is improving, one of the most complex and acutely demanding "special" circulations is the enhanced maternal system to support fetal development. The Barker hypothesis proposes that fetal development within a hostile gestational environment may predispose/program future sensitivity. Therefore, the objective of this study was 2-fold: (1) to determine whether maternal ENM exposure alters uterine and/or fetal microvascular function and (2) test the Barker hypothesis at the microvascular level.

STUDY DESIGN: Pregnant (gestation day 10) Sprague-Dawley rats were exposed to nano-titanium dioxide aerosols (11.3 $\pm$ 0.039 mg/m^3/hr, 5 hr/d, 8.2 $\pm$ 0.85 days) to evaluate the maternal and fetal microvascular consequences of maternal exposure. Microvascular tissue isolation (gestation day 20) and arteriolar reactivity studies (<150 μm passive diameter) of the uterine premyometrial and fetal tail arteries were conducted.

RESULTS: ENM exposures led to significant maternal and fetal microvascular dysfunction, which was seen as robustly compromised endothelium-dependent and -independent reactivity to pharmacologic and mechanical stimuli. Isolated maternal uterine arteriolar reactivity was consistent with a metabolically impaired profile and hostile gestational environment that impacted fetal weight. The fetal microvessels that were isolated from exposed dams demonstrated significant impairments to signals of vasodilation specific to mechanistic signaling and shear stress.

CONCLUSION: To our knowledge, this is the first report to provide evidence that maternal ENM inhalation is capable of influencing fetal health and that the Barker hypothesis is applicable at the microvascular level.

Key words: Barker hypothesis, engineered nanomaterials (ENM), microvascular

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Anthropogenic engineered nanomaterials (ENMs) are manufactured specifically for their unique properties at the nanometer scale (<100 nm in 1 dimension).[1,2] Although their applicability may appear infinite, significant resources have been committed to focus ENM development on engineering and biomedical applications.[3] ENMs have already impacted public health through diverse daily uses (eg, surface coatings, cosmetics, food, drug delivery systems, and implantable medical devices). In many of these applications, adult toxicities have been observed; however, the fetal consequences of maternal exposure to ENM are essentially unknown.

Fetal toxicity and the genetic basis of adult disease are an initiative within the National Institute of Environmental Health and Safety.[4] The general understanding of adult cardiovascular ENM toxicity is modest to good[5]; yet, the maternal and fetal consequences of maternal ENM exposures during gestation are unknown. The "Barker hypothesis" proposes that the association between retarded growth and cardiovascular disease is due to chronic physiologic and metabolic effects that are imposed on a fetus by a hostile gestational environment.[6,7] Limited animal and in vitro studies suggest that maternal ENM exposure has direct consequences on the uterus, placenta, and fetus.[8-11] Nanomaterial influence on any of these tissues can have dire consequences on maternal and/or fetal health. Long et al[8] evaluated the influence that direct nano-sized titanium dioxide (TiO_2) exposure would have on rat neuronal cell cultures and revealed rapid damage to neurons at low concentrations. Blum et al[9] found cadmium oxide within the uterine and placental tissue after maternal inhalation

From the Center for Cardiovascular and Respiratory Sciences and the Department of Physiology and Pharmacology, West Virginia University School of Medicine, Morgantown, WV.

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Reprints: Timothy R. Nurkiewicz, PhD, Center for Cardiovascular and Respiratory Sciences, 1 Medical Center Dr., Robert C. Byrd Health Sciences Center, West Virginia University, Morgantown, WV 26506-9105. tnurkiewicz@hsc.wvu.edu.

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that resulted in associated maternal weight gain and impaired fetal and neonatal growth. Injected ENMs have been shown to reach the uterus easily and stimulate uterine atrophy.[10] Similarly, intravenous maternal ENM exposure also compromises gestation by causing early miscarriage, placental vascular lesions, and fetal malformations that are linked to reactive oxygen species production.[11] Taken together, these findings have a common component, uterine microvascular function that, if altered, may contribute to the generation of a hostile gestational environment.

The most striking observations are structural and functional fetal abnormalities after maternal ENM exposure: impaired implanted fetal resorption (principally in the late gestation), reduction in the number of live fetuses delivered,[12] impaired postnatal growth and weight gain,[9,11] and generalized neurophysical deficits or neurobehavioral alterations in the offspring.[10,13,14] These proof-of-principle studies have shed light on an extremely important issue by injecting high ENM doses to produce significant repeatable results. The functional microvascular ramifications from the maternal or fetal perspective have never been studied in these regards. Furthermore, few studies have focused on the most likely route of ENM exposure: inhalation. It remains to be determined whether ENMs exert a biologic influence through targeting maternal tissues or cross the maternal-fetal barrier to stimulate direct effects on the fetus. Animal studies that evaluate the mechanisms that affect microvascular outcomes must be explored.[15,16]

The microcirculation is the principal level of the vasculature for a host of physiologic parameters that include growth, metabolism, peripheral resistance, tissue perfusion, nutrient/waste exchange, permeability, and leukocyte trafficking. Virtually every pathologic development has a microvascular origin and/or consequence. Our research program has advanced a generally good understanding of the systemic consequences of inhaled ENMs in the coronary and skeletal muscle microcirculations[5,17,18]; however, no work has been done in regards to pregnancy. For example, only recently has environmental particulate matter (air pollution) inhalation been associated with maternal blood pressure disturbances and low birthweights.[19-21] ENMs differ significantly from particulate matter because of their homogeneous composition, unique properties that are associated with their small size, and potential toxicities that are associated with intentional exposures.

Functionally, the microcirculation acts to regulate blood flow distribution while protecting downstream tissues from high arterial pressures and blood flow rates: roles that are crucial for fetal health and survival of a pregnancy. Precise maintenance of blood flow, within an environment of profound remodeling and growth, is paramount for maternal health and fetal development. ENM exposure has been shown to impair normal microvascular reactivity and function[22] in a range of vascular beds, including heart[17,18] and skeletal muscle.[23,24] It is reasonable to speculate that maternal ENM exposure may also influence normal uterine function and lead to a hostile gestational environment, which is capable of impairing fetal microvascular reactivity. Therefore, the purpose of this study was to test the Barker hypothesis from a microvascular prospective.

Materials and Methods

Animal model

Sprague Dawley rats (female, 250-275 g; male, 300-325 g) were purchased from Hilltop Laboratories (Scottsdale, PA). Rats were housed at West Virginia University with food and water provided ad libitum and acclimated for at least 72 hours before use or mating. Females were monitored before breeding to ensure estrus, at which time each female was placed with an individual male. Female rats were then smeared every 12 hours to verify breeding by the presence of sperm. To ensure that all methods were performed humanely and with regard to alleviation of suffering, all procedures were approved by the Institutional Animal Care and Use Committee of the West Virginia University. To increase the likelihood of experimental success and acquisition of viable fetal tissue, rats were placed within the inhalation facility after gestational day 10; if there was maternal exposure before this point, there was a greater chance of reduced litter numbers and ischemic regions within the uterus.

ENM

Nano-TiO_2 powder (aeroxide TiO_2; Evonik Corporation, Parsippany, NJ) is a mixture composed of anatase (80%) and rutile (20%) TiO_2, with a primary particle size of 21 nm and a surface area of 48.08 m^2/g.[24-26] We prepared the nano-TiO_2 for aerosolization by drying, sieving, and storing the powder.[23,24]

Inhalation exposure

We previously reported and described the nano-aerosol generator and exposure system that we used for the current experiments (US patent no. 13/317,472).[23,24,27,28] Briefly, the system was developed specifically for rodent particle inhalation exposures. The apparatus was developed with a vibrating fluidized bed, a Venturi disperser (Vaccon, Medway, MA), cyclone separator, impactor and mixing device, an animal housing chamber, and real-time monitoring devices with feedback control. Aerosols are generated by allowing an air stream to pass through the vibrating fluidized bed and into the Venturi vacuum pump, drawing air and the nano-TiO_2 as it passes. Aerosols enter the cyclone separator, which is gated to remove agglomerates of >400 nm at an input blood flow rate of 60 L/min of clean dry air before entering the exposure chamber.

Size distribution, mean aerodynamic diameter, and relative mass concentration of the aerosols were monitored in real time (Electrical Low Pressure Impactor; Dekati, Tempere, Finland); the particle size distribution was also measured in real time with a Scanning Mobility Particle Sizer device (TSI Inc, St. Paul, MN). Once the steady-state aerosol concentration was achieved, exposure duration was adjusted to achieve a daily calculated deposition of 45 ± 2 μg. Animals were placed within the