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PVP formulated fullerene (C60) increases Rho-kinase dependent vascular tissue contractility in pregnant Sprague Dawley rats

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ABSTRACT

Pregnancy is a unique physiological state, in which C60 fullerene is reported to be distributed in both maternal and fetal tissues. Tissue distribution of C60 differs between pregnant and non-pregnant states, presumably due to functional changes in vasculature during pregnancy. We hypothesized that polyvinylpyrrolidone (PVP) formulated C60 (C60/PVP) increases vascular tissue contractility during pregnancy by increasing Rho-kinase activity. C60/PVP was administered intravenously to pregnant and non-pregnant female Sprague Dawley rats. Vascular responses were assessed using wire myography 24 h post-exposure. Increased stress generation was observed in uterine artery, thoracic aorta and umbilical vein. Rho–Rho-kinase mediated force maintenance was increased in arterial segments from C60/PVP exposed pregnant rats when compared to PVP exposed rats. Our findings suggest that intravenous exposure to C60/PVP during pregnancy increases vascular tissue contractility of the uterine artery through elements of Rho–Rho-kinase signaling during late stages of pregnancy.

1. Introduction

A nano-object is a material with one, two, or three external dimensions in the size range from approximately 1 to 100 nm. These nanomaterials are manufactured in different forms and used in various commercial, biomedical and research technological applications. Fullerenes are carbon based nanoparticles comprised entirely of carbon, in the form of a hollow sphere composed of linked hexagonal carbon rings. C60 fullerenes are composed of 60 carbon atoms and have an average diameter of 0.7–1.0 nm. Engineered fullerenes and their derivatives are designed for various industrial and biomedical applications increasing the probability of occupational, therapeutic and/or environmental exposure. The unregulated exposure to engineered nanomaterials, like C60, may impact human health, much like that described for exposure to particulates in ambient air associated with adverse cardiovascular effects. However, there is less known of the effects of exposure to nanoparticles on vulnerable life stages such as pregnancy. Intratracheal delivery of C60 fullerene is reported to translocate through the alveolar capillary barrier by transcytosis [1], resulting in access to the vascular compartment and to their deposition in endothelial cells. Alternatively, fullerenes and their derivatives used for different biomedical applications such as drug/gene delivery and as contrast agents in diagnostic imaging [2,3] may be introduced directly into the vascular compartment. While the site and extent of C60 distribution within the pulmonary and extra-pulmonary tissues depends on the size of particle agglomerates and route of exposure, once exposed and distributed into the circulation C60 has a very low clearance from the body, remaining in organs/tissues as long as 180 days post-exposure [4,5]. Considering its size and penetrability to tissues and subcellular locations [6], C60 can potentially alter intracellular signaling pathways in cells associated with the vascular walls and impact vascular function.

Abbreviations: 5HT, serotonin; Ach, acetylcholine; ANG II, angiotensin II; BAL, bronchoalveolar lavage; C60, fullerenes with 60 carbons; C60/PVP, polyvinylpyrrolidone formulated C60; DBP, diastolic blood pressure; GD, gestational day; EF, ejection fraction; HR, heart rate; MBP, mean blood pressure; NP, non-pregnant; P, pregnant; PE, phenylephrine; PSS, physiological saline solution; PVP, polyvinylpyrrolidone; RAEC, rat aortic endothelial cells; SBP, systolic blood pressure.

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The current evidence on toxicity of fullerenes and their derivatives on different cell types and tissues are controversial. Fullerene C60 has been described as a molecule with antioxidant properties (*i.e.* a free radical sponge) and as a pro-inflammatory agent [7–10]. A commonly used coating/suspension medium for C60 is polyvinylpyrrolidone (PVP), a water soluble polymer reported to be physiologically inert [11], but the PVP-C60 combination is reported to be toxic in some studies [12]. Non-ionic surfactants such as Tween and Triton provide better solubilization efficacy than PVP for C60 in micellar solutions [13]. However, these agents are more toxic considering their use for intravenous delivery as they hamper the antioxidative properties of C60. We chose to formulate C60 with PVP for our exposure studies as reactive oxygen species quenching ability of C60 is maintained with PVP, despite the moderate solubilization power [13].

With expanding use of C60 based products, it is essential to understand how C60 exposure could potentially influence maternal and fetal vascular tissue contractility, as they can be used to identify both adverse effects and new mechanisms that can be utilized for biomedical applications. Any such impact on vascular contractility can be crucial in life stages such as pregnancy as blood supply to fetus/es can be altered affecting intrauterine growth. Nanoparticle exposures in non-pregnant life stages are known to change vascular reactivity of different vascular beds [14,15]. The extensive proliferative and remodeling environment within the uterine and placental vasculature, particularly during late stages of pregnancy, may make this state more vulnerable to effects of nanoparticle exposure. Pregnancy is a unique physiological state in which the uterine vasculature undergoes outward hypertrophic remodeling influenced by both systemic hormonal changes and localized variations in uterine circulation, which are dependent on the vascular location and stage of pregnancy [16]. The normal physiological changes in uterine vasculature during pregnancy involve enhanced vasodilatation, mediated by augmented basal production of endothelium derived dilator factors including nitric oxide [16,17]. In contrast, Rho kinase activity, a potent pro-contraction process [18] is diminished in normal pregnancy [19] and we suggest may be a potential target for explaining changes in the contractile responses of vessel tissues following nanoparticle exposure.

The evidence for ultrafine particulate matter exposure and its association with pregnancy induced hypertension and low birth weight [20,21] suggest that nanoparticles such as C60 exposure may also have adverse effects on pregnancy as their smaller size and large surface area to mass ratio may increase the possibility of biological and chemical interactions within the vascular system. Following intravenous administration in pregnant rats carbon-14 labeled C60 was reported to distribute to both maternal and fetal organs [22] and such fetal distribution is also reported with maternal exposure to other nanoparticles [23,24]. The Sumner et al. study also reported differences in the distribution pattern of radiolabelled C60 between pregnant and non-pregnant life stages [22], which is presumed to be due to changes in vascular reactivity of various vascular beds during pregnancy. The distribution kinetics of nanoparticles can influence both maternal and fetal vascular function [25], embryogenesis, cellular signaling, inflammation, cell cycle, lipid metabolism [26] fetal growth and malformations [23]. The effects of C60 exposure on maternal/fetal vascular reactivity have not been extensively investigated despite the possibility of occupational, general environmental and therapeutic/diagnostic exposures during pregnancy.

Our interests were to identify how PVP formulated C60 (C60/PVP) exposure affects the vascular responses during pregnancy and whether the resulting changes could impact intrauterine fetal growth to identify the potential need of further developmental toxicity assessments of these nanoparticles. We hypothesized that exposure to PVP formulated C60 *via* intravenous administration would enhance the contractile responses of uterine and placenta derived blood vessels during pregnancy, potentially reducing fetal blood supply. In addition, we assessed the contribution of RhoA–Rho kinase pathway as a potential mechanism underlying the changes in maternal vascular tissue contractility following C60 exposure.

2. Materials and methods

2.1. Characterization of nanoparticles and suspensions

C60 was commercially procured from Sigma–Aldrich (St. Louis, MO, USA, Catalog# 379646) and formulated with polyvinylpyrrolidone (PVP) (Sigma–Aldrich, St. Louis, MO, USA; Catalog# 234257) at RTI International (Research Triangle Park, NC, USA). In order to enable the suspension of these non-functionalized hydrophobic particles in physiologically compatible media, they were formulated with PVP, as described previously [22,27]. The hydrodynamic diameter (*Z*-average) and the zeta potential of the C60/PVP dosing suspensions were determined using a Malvern Zetasizer NanoZS (Malvern Instruments, Worcestershire, UK) with a 633 nm laser source, a detection angle of 173 degree, and a clear disposable sample cell. DLS measurements were carried at 25 °C using the following protocol: (1) 1st size determination; (2) zeta potential measurement; (3) 2nd size determination. The time elapsed between the two size determinations was approximately 8 min and the average particle size was calculated by averaging 1st and 2nd measurement results. The hydrodynamic particle sizes were measured at following time points: (1) right after sample preparation; (2) 38 min after sample preparation (mimic sample dosing period). The measurement at 38 min after the preparation was done to confirm that the suspensions remained stable at the time of delivery to rats.

2.2. Sprague Dawley rats

Ten to twelve week old timed-pregnant and non-pregnant female Sprague Dawley rats were purchased from Charles River Laboratories (Raleigh, NC, USA) to study the effects of *in vivo* C60 exposure on dame vascular tissue contractility and the changes in the fetal weight. The determination of pregnancy was by observation of the vaginal plug, the plug date was considered to be day zero of gestation and the rats arrived in the animal facility within 9–12 days of gestation onset. All rats were individually housed under 12 h light/dark cycles with standard rat chow and water provided *ad libitum*. Body weights were monitored (two readings taken three days apart) during a one week acclimation in Department of Comparative Medicine's Animal Facility at East Carolina University (ECU) to ensure progression of the pregnancy. In our preliminary experiments, two gestational age groups were evaluated: gestational days (GD) 14–16 and GD 17–19, to represent the maternal early third trimester of a human pregnancy and the early fetal stage of the rodent pregnancy. Following a detailed regression analysis, significant changes in vascular reactivity with C60 exposure were seen only in the late gestational stage (GD 17–19) group and all subsequent studies were done on this late gestational age group. All studies were designed to investigate potential mechanism of vascular dysfunction associated with C60 exposure and not specifically to investigate longitudinal or ontological change of the vascular responses with gestation. In addition, a third group composed of age-matched non-pregnant Sprague Dawley was also studied. All animal handling procedures were approved by ECU Institutional Animal Care and Use Committee.