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Prenatal cigarette smoke exposure: Pregnancy outcome and gestational changes in plasma nicotine concentration, hematocrit, and carboxyhemoglobin in a newly standardized rat model

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Abstract

Epidemiological studies support an association between perinatal cigarette smoke (CS) exposure and a number of severe pre- and postnatal complications. However, the mechanisms through which CS enhances such risks largely remain unknown. One of the reasons for our inability to discover such mechanisms has been the unavailability of a clinically relevant and physiologically concordant animal model. A number of studies have previously used nicotine (Nic) as surrogate for CS. We sought to (1) establish the amount of CS exposure to achieve plasma Nic concentrations observed among moderate to heavy smokers (20–60 ng/ml), (2) investigate the temporal changes in plasma Nic concentrations, carboxyhemoglobin, and hematocrit with advancing pregnancy, and (3) elucidate the effects of CS exposure on pregnancy outcome. Pregnant Sprague–Dawley rats were exposed to various doses of CS or room air (Sham) from days 6 to 21 of gestation. Exposure to 6000 ml/day of CS led to very high plasma Nic concentrations and increased maternal and fetal mortality ($P < 0.001$). The plasma Nic concentrations remained higher than those observed in moderate smokers until the CS dose was reduced to 1000 ml/day and showed dose-dependent temporal changes with advancing gestational age. Significant increases in carboxyhemoglobin and hematocrit were observed in the CS group as compared with the Sham group ($P < 0.001$). In addition, prenatally CS exposed fetuses had lower birth weight as compared with the Sham group ($P = 0.04$). Our current study establishes a newly standardized and physiologically relevant model to investigate the mechanisms of CS-mediated adverse effects during the critical period of fetal development.

Keywords: Low birth weight; Newborn; Nicotine kinetics; Sudden infant death syndrome (SIDS); Maternal smoking

Introduction

At present, over 1.1 billion people worldwide are exposed to mainstream cigarette smoke (CS), and 5 million die annually due to tobacco related diseases (Ezzati and Lopez, 2003). If unabated, the mortality rate is expected to double by year 2010 (Editorial, Nature Medicine, 2005). CS exposure during pregnancy is by far the most common known chemical insult to the developing fetus. Perinatal studies have shown an association between maternal smoking and adverse maternal, fetal, and neonatal outcomes (Cnattingius, 2004), including spontaneous abortions, placental abruption, placenta previa (Zdravkovic et al., 2005), fetal death (Yuan et al., 2005), preterm delivery, low birth weight, reduced postnatal growth, aberrant pulmonary structural and functional changes, sudden infant death syndrome (SIDS), and behavioral abnormalities (Centers for Disease Control and Prevention, 2004; Johnson et al., 2000; Mathews et al., 2004). Despite the advocacy of supine sleeping position, SIDS remains the leading cause of death between 1 month and 1 year of age and is generally considered to result from impairment of cardiorespiratory control including arousal (Mathews et al., 2004). The precise pathophysiological events leading to death, however, remain unknown.

Although a large number of epidemiological studies have suggested CS exposure to be a strong and independent risk factor for various pre- and postnatal complications (Cnattingius, 2004), little is known about the biologic mechanisms through which CS

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enhances such risks including SIDS. One of the reasons for our inability to discover the mechanisms has been the unavailability of physiologically and pharmacologically concordant animal model to investigate the adverse effects of prenatal CS exposure during the critical period of fetal development.

Due to its well-established effects on cardiovascular dynamics and neurotoxicity, nicotine (Nic) has been used as a surrogate for CS (Xu et al., 2001). Although Nic is an important and addictive constituent of CS, it is only one of the 4700 chemicals present in a lighted cigarette (Smith and Fischer, 2001). Implantation of osmotic mini-pumps did overcome a number of limitations associated with oral or subcutaneous Nic administration (Murrin et al., 1987). However, continuous infusion of Nic, as used in a number of studies (Slotkin, 2004; Xu et al., 2001) is not the usual method of CS exposure and lacks the peaks and troughs of plasma Nic concentrations normally observed among human smokers (Benowitz et al., 1982; Hukkanen et al., 2005).

Attempts have been made to investigate the mechanisms of CS exposure on fetal growth and development (Bassi et al., 1984; Younoszai et al., 1969; Reznik and Marquard, 1980). In one such study (Bassi et al., 1984), pregnant rats were exposed to CS obtained from three simultaneously lit cigarettes, 17 times per day at 30-min intervals. The carboxyhemoglobin (COHb) saturation in the maternal blood ranged from 10 to 20%, which is 3-fold higher than the values observed among moderate smokers (Benowitz and Jacob, 2000). Furthermore, neither the volume of CS nor plasma Nic concentration was measured. In another study (Younoszai et al., 1969), exposed pregnant rats to a variable number of cigarettes containing as high as 15 mg of Nic per cigarette, which is 6- to15-fold the amount of Nic contained in a standard cigarette (Benowitz and Jacob, 2000). Once again, no Nic concentrations were reported. Reznik and Marquard (1980) administered CS obtained from 30 cigarettes for 7–11 min continuously during a given exposure. The number of exposures varied from 1 to 4 times per day. Although this study showed an adverse effect on fetal growth, no data were provided on plasma Nic concentrations, COHb, or maternal and fetal mortality. Almost all other studies have administered the 35 ml puff volumes recommended by the Smoke Exposure System manufacturers, which can reach near-lethal COHb values and unrealistic plasma Nic concentrations (Coggins, 1998). Whole body CS exposures to mimic environmental tobacco smoke have been given 4–6 h per day (Joad et al., 1995). Therefore, the relevance of these studies has been compromised by the extremely high (1) puff volume, (2) amount of Nic in cigarettes, (3) duration and frequency of exposures, and/or (4) the number of cigarettes per exposure. Equally important is the fact that no data are available on the sequential changes in plasma nicotine concentrations, carboxyhemoglobin, or hematocrit at various gestational ages. Since the metabolism of Nic is highly complex and dependent on a number of exogenous and endogenous factors (Hukkanen et al., 2005; Seaton and Vesell, 1993), it is not known whether changes in maternal homeostasis with advancing pregnancy would modify plasma Nic concentrations and its effects on maternal and fetal variables. Thus, despite the significance of the large number of perinatal disorders causally attributable to maternal smoking at a global level, the effects of CS on various maternal and fetal outcomes in a physiologically and pharmacologically relevant and a reproducible model for prenatal CS exposures have not been elucidated.

The specific aims of our current study were (1) to establish the dose–concentration relationship and the daily volume of CS required to achieve the plasma Nic concentrations observed in moderate to heavy smokers, (2) to investigate the temporal changes in plasma Nic concentrations, hematocrit (Hct), and COHb at various gestational ages, and (3) to elucidate the dose-related effects of CS exposure on maternal and fetal mortality and morbidity.

Methods

All studies were carried out in accordance with the guidelines of the Canadian Council on Animal Care, and the experimental protocols were approved by the Animal Care Committee of the University of Calgary. Sprague–Dawley rats were housed individually in breeding cages and mated locally at the University of Calgary. The pregnancy was confirmed by the formation of a vaginal mucus plug, and the first day after mating was considered gestational day 1. The temperature in the facility was maintained at 20 °C with a 12-h light–dark cycle, and the animals had free access to food (Rat Chow, Canadian Lab Diets Inc. Leduc, Alberta) and tap water. The pregnant animals were exposed to CS or room air (Sham) using the Smoke Exposure System.

Smoke exposure system

The commercially available Smoke Exposure System (Tobacco Research Institute, University of Kentucky, Lexington, KY) has three main components: smoke generation, smoke distribution, and animal exposure units. This nose-only exposure system developed for small rodents has the capability of providing both, intermittent mainstream (MS) and/or continuous side-stream (SS) smoke from a single lighted cigarette to 16 animals simultaneously.

The Smoke Generation Unit is comprised of a puffer, pump, and SS collection chamber. The puff volume can be adjusted by regulating the puffer or by calibrating the air/smoke flow through the main vacuum line, waste line, and pump head line. The smoke generated from a single puff was transferred to the smoke distribution units. The smoke distribution unit, in turn, allocated the MS smoke to the exposure blocks where 8 rats were exposed to CS or room air simultaneously. In our current study, one puff was produced every minute with each puff lasting 2.4 s. During the Sham exposures, the MS smoke generation pump was replaced by a Sham pump, which provided airflows similar to those given during CS exposures. The Sham pump, which is identical to the CS pump, was never used to provide CS in order to avoid an inadvertent exposure of the CS constituents to the Sham groups.

The Smoke Exposure System was calibrated twice daily using a flow meter, and a control run (without animals) was performed to monitor its performance. All the tubing, filters, valves, and connecting components were cleaned and changed daily. Research cigarettes (2R1; Tobacco Research Institute, University of Kentucky) with a resistance to draw of 8.94 cmH_2O, 660 static burn s/40 mm, 85 mm long, 25 mm circumference, paper porosity of 47.6 s/50 cm^3 and containing 2.45 mg of Nic were used. The reference cigarettes were placed in a plastic airtight bag in a standard laboratory refrigerator until used for research. This procedure was suitable to hold the proper moisture level in the cigarettes and was recommended by the Tobacco and Health Research Institute, University of Kentucky. In addition, before the cigarettes were used for research, they were exposed to room temperature for approximately 15 min. The contents of the reference research cigarettes conform to the US Federal Trade Commission standards.

Experimental design

All experiments were performed on time-dated pregnant Sprague–Dawley rats weighing 250–270 g. Once the puff volume and frequency of puffs were