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Effects of prenatal exposure to cigarette smoke on biochemical parameters of the blood of adult rat offspring

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Prenatal cigarette smoke (CS) exposure during pregnancy is known to be associated with many severe prenatal and postnatal developmental abnormalities. The present study investigates serum biochemical parameters of adult rat offspring exposed prenatally to CS. Female white albino rats were exposed to CS during pregnancy 3 times/day (each 15 minutes) from gestational day 0 to parturition. Serum biochemical parameters were measured in adult male and female rat offspring (9-10 weeks in age) exposed prenatally to CS. Maternal CS exposure during gestation had no significant ($P > 0.1$) effect on serum biochemical parameters, including glucose, total proteins, albumins, globulins, albumins/globulins ratio, cholesterol, triglycerides, high density lipoproteins (HDL), low density lipoproteins (LDL), creatinine, bilirubin, urea, uric acid, alanine aminotransferase (ALT), alkaline phosphatase (ALP) in the blood of adult male and female rat offspring. However, prenatal CS exposure significantly ($P < 0.05$) decreases serum aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) in the blood of adult male and female rat offspring. Results demonstrated that CS exposure during gestation significantly decreases serum AST and GGT in the blood of adult male and female rat offspring.

Keywords: Pregnant rats; cigarette smoke; blood; maternal smoking; biochemical parameters; adult rat offspring

INTRODUCTION

Maternal cigarette smoking during pregnancy has long been associated with many severe pre- and postnatal developmental abnormalities in human fetus and infant (Kahn et al. 1994; Blair et al. 2006; Moon et al. 2007; Urato et al., 2008; Koshy et al. 2010; Votavova et al. 2012). Prenatal cigarette smoke (CS) or nicotine exposure significantly increases the risk of perinatal complications, including morbidity and mortality, preterm delivery, spontaneous abortion, low birth weight, intrauterine growth restriction, sudden infant death syndrome, impair immune responses, reduced fertility and kidney underdevelopmen (Lieberman et al. 1994; DiFranza and Lew 1995; Florek and Marszalek, 1999; Haustein, 1999; Andres and Day, 2000; Blake et al. 2000; Dempsey and Benowitz, 2001; Duncan et al. 2009; Mansen et al.2010; Taal et al. 2011). In addition, human and animal studies have shown that fetal exposure to maternal cigarette smoking or nicotine during gestation is associated with adverse long-term postnatal health disorders in the offspring, such as neurobehavioral and cognitive deficits, obesity, cardiovascular and respiratory diseases, and impaired glucose homeostasis (Blake et al. 2000; Schuz et al. 2001; Landau, 2001; Gilliland et al. 2003; Toschke et al. 2003; Oken et al. 2005; Voiget et al. 2006; Bruin et al., 2007; Pachlopnik et al. 2007; Ino 2009; Koshy et al. 2010; Mezzacappa et al., 2011; Högberg, 2012; Pendlebury et al. 2012; Jaddoe et al. 2014).

Animal studies have demonstrated that CS or

nicotine exposure during pregnancy is associated with significant prenatal (Seller and Bnait, 1995; Gao et al. 2005) and long-term postnatal developmental outcomes in the offspring, such as neurobehavioral defects, increased body weight, impaired glucose metabolism, hyperinsulinemia, dyslipidemia, fertility, increased brain inflammation, oxidative stress, structural alterations in the brain, tumor Susceptibility, chronic cardiovascular, respiratory and kidney dysfunction (Newman et al. 1999; Ng et al.2000, 2006; Roy et al. 2002; Li, 2003; Singh et al. 2003; Pausova et al. 2003; Gao et al. 2005; Holloway et al., 2006; Bruin et al., 2007; Yang et al. 2007; Thomas et al. 2007; Ng and Zelikoff, 2008; Al-Odat et al. 2014; Yochum et al. 2014; Chan et al. 2016; Fan et al. 2016).

CS is known to be a complex mixture of large number of highly toxic chemicals, including nicotine, polycyclic aromatic hydrocarbons, carbon monoxide, tar, benzene, phenol and heavy metals, such as cadmium, arsenic and lead that can cause severe damage to fetal tissues (Boadi et al. 1992; Czekaj et al.2002; Dai et al. 2015). Human and animal studies have shown that many lipophilic constituents of CS, such as nicotine, cotinine, a metabolite of nicotine, and carbon monoxide rapidly cross the placental tissue into the developing embryo (Donnenfeld et al. 1993; Lambers and Clark 1996; Jauniaux et al. 1999; Perera et al., 2004; Pichini et al., 2000; Lips et al., 2005), causing severe pre- and postnatal developmental complications (Lambers and Clark 1996; Florek and Marszalek, 1999; Pichini et al. 2000; Perera et al. 2004). Nicotine, cotinine and cadmium have been identified in fetal blood, amniotic fluid, saliva and urine of newborns, and in breast milk during lactation and blood of smoking mothers (Boyle, 1993; Lambers and Clark, 1996; Pichini et al. 2000; Berlin et al. 2010; Mansen et al., 2010; Wrzesniak et al. 2016), and in fetal blood of rodents (Carmines et al. 2003; Holloway et al. 2006; Bruin et al. 2007). Humans and animal studies have demonstrated that nicotine and carbon monoxide can cause a direct increase in placental vascular resistance and decrease fetal blood flow (Bruner and Forouzan, 1991; Clark and Irion, 1992; Donnenfeld et al. 1993; Lambers and Clark, 1996; Sastry et al. 1998; Albuquerque et al. 2004). The reduction in placental blood flow causes severe decrease in fetal nutrients and oxygen supply to the developing fetus (Soothill et al. 1996).

In addition, maternal cigarette smoking during pregnancy stimulates the release of catecholamines from adrenal glands and sympathetic autonomic nerves which consequently limits the placental blood flow, creating a state of hypoxia and malnutrition to the fetus (Ernest et al., 2001). The aim of the present study was to examine the effect of maternal cigarette smoking during pregnancy on serum biochemical parameters of the blood of adult rat offspring.

MATERIALS AND METHODS

Animals

Experiments were carried out on mature female albino rats 9-10 week in age (body weight 180-200 g). The animals were housed individually in standard conditions in polycarbonate cages with wire mesh tops, and allowed to acclimatize for one week. The rats were provided with standard laboratory chow and tap water *ad libitum* except during CS exposure. Animal room was maintained at 20 – 25°C. Care and handling of the animals were performed in accordance with the Principles of laboratory animal care. During mating, a single fertile male rat was paired with two females overnight. Pregnancy was confirmed the next morning by examining the female rats for the presence of sperms in the vaginal smears (considered gestational day 0). At the end of mating period, the males were removed. For comparison, a second non-exposed group was used as control.

Cigarette smoke exposure

The procedure used for CS exposure has been described (Shakhanbeh, 2016). Pregnant females of CS exposure group were daily exposed to CS via whole-body inhalation (3 times/day, each 15 minutes at 4 hours intervals) from gestational day 0 until parturition (Gestational Day 22). CS was generated from the burning of filtered cigarettes using a smoking device (designed in Neurobiology laboratory, Mutah University), and delivered in the form of puffs (each 35 ml) at regular intervals (one puff/min, and puff duration of 2 seconds). There were no deaths or any other abnormal signs in the pregnant females associated with CS exposure. The control group was subjected to the same experimental conditions of the CS exposure group, but without exposure to CS. Food consumption and body weight gain of pregnant females of CS exposure group were similar to that of pregnant females of the control group. Newborn pups were kept with their mothers until weaning.

After weaning, male and female offspring of CS exposure group and control group were separated and kept in the animal house. Serum biochemical parameters of the blood were measured in adult male and female rat offspring (9-10 weeks in age) of both groups.

Serum biochemical parameters analysis

Blood samples (5 ml) were collected via cardiac puncture under light dimethyl ether anesthesia from fasting adult male and female rat offspring of CS exposure group and controls. The blood samples were allowed to clot at 4 °C for 30 minutes, centrifuged at 3000 rpm for 5 minutes, and stored at -20°C until assayed. Analysis of serum biochemical parameters, including glucose, total proteins, albumins, globulins, cholesterol, triglycerides, HDL, LDL, creatinine, bilirubin, uric acid, ALT, AST, ALP and GGT were measured using commercially available kit of Rouch, Germany, and using the automatic analysis apparatus Cobes. The serum globulin was calculated by subtracting serum albumin from serum total protein levels.

Statistical analysis

Statistical analysis was performed by Student's *t*-test for independent samples using Excel Software. The level of significance of all tests was set at $P < 0.05$, and the results were expressed as mean ± standard error (SE).

RESULTS

Serum biochemical parameters of the blood of adult rat offspring after prenatal CS exposure

Serum biochemical parameters of the blood of adult male and female rat offspring after prenatal CS exposure group and non-exposed controls are shown in Table 1. Prenatal CS exposure had no significant ($P > 0.1$) effect on serum biochemical parameters, including glucose, total proteins, albumins, globulins, albumins/globulins ratio, cholesterol, triglycerides, HDL, LDL, creatinine, bilirubin, urea, uric acid in the blood of adult male and female rat offspring.

Serum enzyme levels of the blood of adult rat offspring after prenatal CS exposure

Serum enzyme levels of the blood of adult male and female rat offspring after prenatal CS exposure and non-exposed controls are shown in table 2. Prenatal CS exposure had no significant ($P > 0.1$) effect on serum alanine aminotransferase and alkaline phosphatase levels in the blood of male and female adult rat offspring. However, prenatal CS exposure significantly ($P < 0.05$) decreases serum AST and GGT levels in the blood of male and female adult rat offspring as compared with non-exposed controls. The percentage decrease in AST in males and females was 17.14% and 25.34% respectively. In addition, the percentage decrease in GGT in males and females was 76.25% and 62.32% respectively.

DISCUSSION

The present study investigates serum biochemical parameters of adult rat offspring exposed prenatally to maternal cigarette smoking. The results demonstrated that prenatal CS exposure had no significant ($P > 0.1$) effect on serum biochemical parameters, including glucose, total proteins, albumins, globulins, albumins/globulins ratio, cholesterol, triglycerides, HDL, LDL, creatinine, bilirubin, urea, uric acid, alanine aminotransferase, alkaline phosphatase in adult rat offspring. However, previous animal studies have shown that maternal CS exposure induced increased albumin/creatinine ratio accompanied by severe structural abnormalities in the kidneys of adult mice offspring (Al-Odat et al., 2014). This might be due to chronic fetal hypoxia and/or ischemia induced by carbon monoxide and nicotine of the CS, causing reduction of the uteroplacental blood flow during gestational period (Bruner and Forouzan, 1991; Clark and Irion, 1992; Donnenfeld et al. 1993; Lambers and Clark, 1996; Sastry et al. 1998; Albuquerque et al. 2004) which may affect the development of fetal hematopoietic tissues, such as liver and spleen. This is supported by the findings of Diniz et al. 2013 who showed that severe structural abnormalities in the liver and spleen of adult mice offspring exposed prenatally to CS.

The results of the present study showed that maternal cigarette smoking during pregnancy significantly ($P < 0.05$) decreases liver enzymes aspartate aminotransferase and gamma glutamyl transferase in adult males and females rat offspring (table 2). This decrease in liver enzymes is in agreement with the finding of Diniz et al., 2013 who described severe structural deficits in the liver and spleen of adult mice offspring exposed prenatally to CS.

Table 1:Serum biochemical parameters of the blood of adult male and female rat offspring after prenatal CS exposure and non-exposed controls.

Biochemical Parameters	Males		Females	
	CS exposure	Controls	CS exposure	Controls
Glucose (mg/dL)	97.96 ± 3.59 (n = 20)	92.11 ± 3.26 (n =24)	94.95 ± 4.56(n =20)	102.35 ± 4.16(n = 19)
Total proteins (g/dL)	6.08 ± 0.06 (n =14)	6.08 ± 0.07 (n = 16)	6.29 ± 0.08 (n = 14)	6.30 ± 0.11 (n = 15)
Albumins (g/dL)	4.10 ± 0.06 (n = 15)	3.99 ± 0.05 (n = 16)	4.21 ± 0.05 (n = 11)	4.24 ± 0.07 (n = 15)
Globulins (g/dL)	2.01 ± 0.05 (n = 15)	2.08 ± 0.04 (n = 16)	2.14 ± 0.08 (n = 11)	2.05 ± 0.12 (n = 15)
Albumins/Globulins	2.053 ± 0.06 (n = 15)	1.98 ± 0.05 (n = 16)	2.01 ± 0.08 (n = 11)	2.04 ± 0.6 (n = 15)
Cholesterol (mg/dL)	56.41 ± 1.33 (n = 29)	58.63 ± 1.98 (n =38)	57.35 ± 1.4 (n = 28)	59.35 ± 2.37 (n = 40)
Triglycerides(mg/dL)	77.49 ± 4.66 (n = 26)	83.48 ± 4.48 (n =29)	80.58 ± 4.16 (n =27)	86.31 ± 5.81 (n = 35)
HDL (mg/dL)	53.12 ± 1.52 (n = 16)	55.08 ± 2.49 (n =15)	52.70 ± 1.98 (n =15)	52.46 ± 2.73 (n = 11)
LDL (mg/dL)	7.02 ± 0.55 (n = 15)	8.59 ± 0.56 (n = 15)	3.22 ± 0.26 (n = 13)	3.16 ± 0.27 (n = 11)
Creatinine (mg/dL)	0.38 ± 0.03 (n = 20)	0.34 ± 0.02 (n = 24)	0.50 ± 0.04 (n = 25)	0.4 ± 0.03 (n = 30)
Bilirubin (mg/dL)	0.05 ± 0.01 (n = 15)	0.07 ± 0.01 (n = 14)	0.07 ± 0.01 (n = 15)	0.05 ± 0.01 (n = 11)
Urea (mg/dL)	42.86 ± 2.05 (n = 29)	38.49 ± 1.21 (n =33)	42.81 ± 1.47 (n =27)	39.46 ± 1.34 (n = 36)
Uric acid (mg/dL)	1.27 ± 0.12 (n = 29)	1.53 ± 0.10 (n = 30)	1.49 ± 0.19 (n = 23)	1.60 ± 0.12 (n = 30)

HDL: high density lipoproteins, LDL: low density lipoproteins.

Table 2:Serum enzyme levels of the blood of adult males and female rat offspring after prenatal CS exposure.

Serum enzymes	Males		Females	
	CS exposure	Controls	CS exposure	Controls
ALT (IU/L)	40.27 ± 2.10 (n = 20)	43.99 ± 2.30 (n =31)	39.28 ± 2.40 (n=25)	39.19 ± 2.15 (n = 31)
ALP (U/L)	140.24 ± 6.48 (n = 20)	147.77 ± 8.50 (n =20)	118.15 ± 7.74(n =22)	100.72 ± 7.34 (n =15)
AST (IU/L)	117.64 ± 6.40* (n = 23)	141.1 ± 9.87 (n = 32)	111.39 ± 7.45* (n=23)	149.21 ± 12.55 (n=29)
GGT (U/L)	0.66 ± 0.18* (n = 14)	2.78 ± 0.90 (n = 11)	1.59 ± 0.30* (n = 14)	4.22 ± 0.53 (n = 10)

ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, GGT: gamma glutamyl transferase. Significantly different from controls ($^{*}P < 0.05$).

Human studies have shown that cigarette smoking during pregnancy induced decease in several serum protein fractions, increase Cadmium (Cd) concentration and decreases Zink concentration in the blood of smoking pregnant women (Milnerowicz et al. 2000; Wrzesniak et al. 2016). The decrease in protein concentration leads to higher oxidative stress to the mother and fetus. Moreover, the accumulation of Cd in placental tissue may interfere with the transport of gases and nutrients to the developing fetus. In addition, chronic fetal hypoxia and/or ischemia induced by carbon monoxide and nicotine of the CS during gestational period can cause reduction of the uteroplacental blood flow (Donnenfeld et al. 1993; Soothill et al. 1996; Albuquerque et al. 2004) which consequently affects the development of hematopoietic tissues, including liver, spleen and bone marrow (Diniz et al. 2013).

CONCLUSION

Cigarette smoke exposure during gestational period induced permanent significant reduction in serum aspartate aminotransferase and gamma glutamyl transferase in adult rat offspring. However, other serum biochemical parameters, including glucose, total proteins, albumins, globulins, cholesterol, triglycerides, high-density lipoproteins, low-density lipoproteins, creatinine, bilirubin, urea, uric acid, alanine aminotransferase, alkaline phosphatase were normal in adult rat offspring after prenatal CS exposure.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

GS designed the experiments. RR performed the experiments. AA wrote the manuscript and GS reviewed the manuscript. All authors read and approved the final version.

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