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Effects of prenatal exposure to passive cigarrette smoke and nicotine on nitric oxide and blood glucose levels of rats

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ABSTRACT: The effects of prenatal exposure to cigarette smoke or nicotine on plasma nitric oxide and blood glucose level of adult rats was studied in this work.

Fifteen female Wistar rats were divided into 3 groups of five rats each (A-C).Group A rats were exposed to cigarette smoke in an exposure chamber, Group B rats were administered 0.25 mg/kg b.w of nicotine while Group C rats served as the Control. After mating and gestation, litters from each group were randomly subdivided into two groups of 5 neonates each. Group A: A_1 and A_2, Group B:B_1, and B_2,Group C:C_1 and C_2.

Groups A_1, B_1 and C_1 were further administered 50 mg/kg b.w Vitamin C for 4 weeks after birth; Groups A_2 and B_2 and C_2 received nothing .Blood samples were collected from all animals and nitric oxide and blood glucose levels were estimated.

Results show that the neonate s of animals exposed to cigarette smoke had a significantly higher nitric oxide concentration than those not exposed, and a significantly lower blood glucose level(61.00 +2.03 mg/ dL) when compared with the control(75.50 + 3.73 mg/dL).On the other hand prenatal exposure to nicotine neither had a significant effect on nitric oxide concentration(12.50+2.10 µM) when compared with the control(10.75+2.95 µM) nor on blood glucose level (74.17+3.48 mg/dL) and (75.50+3.73 mg/dL)respectively.

The effects of cigarette smoke shown here could not be attributed to the pharmacological activity of nicotine,but may be related to the formation of smoking induced oxidative free radicals,as administration of anti-oxidant,vitamin C,reversed these effects.

Keywords: Nitric Oxide, Blood Glucose Level, Cigarette smoke, Nicotine, Vitamin C, Prenatal exposure

Introduction

Cigarette smoking continues to be a widespread public health problem; of the nearly 1 billion smokers worldwide, half are likely to die of smoking-related diseases. The maintenance of smoking behavior is believed to hinge on the addictive effects of nicotine, mediated predominantly through the actions of the drug at nicotinic acetylcholine receptors, which are distributed throughout many cortical and subcortical brain regions, notably the thalamus and midbrain. Through actions at these receptors, nicotine has widespread effects on the central and peripheral nervous systems. These effects include facilitation of neurotransmitter release from dopaminergic, cholinergic, glutamatergic, serotonergic, and γ-aminobutyric acid-ergic nerve terminals (Jed *et al*, 2003).

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While neonatal mortality has greatly declined through improved paediatric care, unfortunately there seems to be no similar reduction in the incidence of infant morbidity and in the occurrence of childhood developmental disorders of the brain and intellect. The reasons for this are manifold; however, several investigations have now been able to provide ample evidence that pre-term and low birth weight infant carries the major risk of suffering from developmental delay, whether mental or physical. In addition, more serious conditions such as mental retardation, retinopathy of prematurity, broncho-pulmonary dysplasia, brain haemorrhage, spasticity, cerebral palsy, blindness, deafness, autism and epilepsy are a frequent consequence of pre-term birth, which has been linked to cigarette smoking during pregnancy (Crawford, 1993)

The health effects of subchronic exposure to low levels of wood smoke in rats were examined by Yohannes *et al* (2001). They observed that pulmonary functions, especially CO_2 diffusing capacity and pulmonary resistance were somewhat affected in high exposure group. Also mild chronic inflammation and squamous metaplasia were observed in the larynx of exposed groups. The severity of these increased with smoke concentration and length of exposure. Also Xiu *et al* (2005) studied the effects of cigarette smoke on degranulation and nitric oxide (NO) production by mast cells and epithelial cells, and found that exhaled NO is decreased in active and passive smokers, suggesting that it inhibits NO production. Since NO is the most important vasodilator synthesized by blood vessels, this study is designed to examine whether prenatal exposure to cigarette smoke affects plasma NO with alteration of blood glucose level.

Materials and Methods

Animals

Fifteen (15) presumably healthy female rats obtained from the central Animal House, College of Medicine, University of Ibadan were used for this work. The rats weighed between 250-300g and were housed in well ventilated cages(5 animals per cage) in the Animal House of the Department of Physiology ,University of Ibadan.They were exposed to 12 hour light and 12 hour-dark cycle,relative humidity 50-53% and a temperature range of 26-28^{0}C.The animals were fed rat pellets and tap water *adlibitum* and were allowed two (2) weeks of acclimatization.

Ovulation/Mating: Ovulation was induced by administration of stilboestrol – an orally active synthetic estrogen. 0.042 mg/ kg b.w of stilboestrol was administered using an oral cannula at 9:00a.m. Ovulation was confirmed by vaginal smear according to the method described by Marcondes *et al* (2002).

Mating was allowed naturally by introducing a male rat into each group. Estrus cycle occurs every 3-4 days in rats and gestation lasts 21-23 days, with notable pregnancy at about 14 days. Each female rat had between 6 and 15 litters.

Cigarette Smoke Exposure: London king size (menthol) cigarette, produced by London Tobacco Company was used. Each contains 14.9 mg tar and 1.2 mg nicotine. Rats were exposed to smoke from an idling cigarette in the exposure chamber over a period of 30 minutes per day (9.00 a.m-9.30 p.m), from day 0 to day 20 of gestation. Four (4) cigarettes were used for each animal group per exposure per day. Food and water were removed from chambers before start of exposure, and then replaced after exposure.

Nicotine Admistration

Standard nicotine was used. 0.25mg/kg b.w was administered by intramuscular injection daily from day 0 to day 20 of gestation.

Vitamin C Administration

50 mg/kg b.w of vitamin C (by Tuyil Pharmaceutical Company, Ilorin,Kwara State)was administered orally by means of an oral cannula on a daily basis for a period of six(6) weeks,beginning from 4 weeks post-birth.

Estimation of Nitric Oxide (NO)

Blood samples were collected by left cardiac puncture after ether anesthesia, allowed to clot for 15 min and centrifuged at 2000 × g for 10 minutes at room temperature. Serum specimens were portioned into polypropylene tubes. All biological specimens were stored at -10 °C until analyzed.
Plasma Nitric oxide was estimated by the Griess Reagent System (assay). This involves measuring nitrite (NO_2), which is one of two primary, stable and nonvolatile breakdown products of NO. This assay relies on a diazotization reaction that was originally described by Griess in 1879 (Muijsers *et al*, 2000). Absorbance was read at 540nm in a spectrophotometer (Pharmacia Biotech,Uppsala Sweden).Average absorbance value of each experimental sample was calculated and its concentration determined by comparison (reading off) to the Nitrite Standard reference curve.

Determination of Blood Glucose Level (BGL)

Fasting BGL was estimated ten (10) weeks post-birth. Blood samples (20 l) were collected from the tail-tips of conscious rats after 12 hours overnight fast. Glucometer (Acu-chek® Johnson-Johnson,California, USA) and compatible glucometer strips were used for the determination of blood glucose levels.

Experimental Design

Fifteen (15) female Wistar rats were divided into 3 groups (A-C) of 5 rats each. After mating and confirmation of pregnancy, Group A rats were exposed to cigarette smoke, Group B rats were administered nicotine while Group C rats served as the control. After parturition, neonates of the rats were subdivided as follows: Group A: A_1 and A_2; Group B:B_1 and B_2;Group C: C_1 and C_2.
Groups A_1, B_1 and C_1 further received 50 mg/kg b.w of vitamin C while Groups A_2, B_2 and C_2 did not. Animals were checked once in the morning and once in the late afternoon at least 6 hour apart 7 days a week for any clinical signs of abnormality, morbidity, or mortality as outlined by Jaci *et al* (1997).
All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (American Physiological Society, 2002) and were approved by the Departmental Committee on the Use and Care of Animals.

Statistical Analysis

Data are expressed as mean ± SEM and analysed using the student's t-test and ANOVA where necessary.$P< 0.05$ was accepted as significant.

Results

Effect of Cigarette smoke and nicotine on Nitrite Concentration

Intrauterine exposure to passive cigarette smoke had a significant effect on plasma NO. Offspring of pregnant rats exposed to passive CS had a statistically significantly higher NO concentration (Table 1).Results showed no significant difference between Groups A_1 and A_2, indicating the ameliorative effect of vitamin C administration.

Table 1:Effect of cigarette smoke on nitrite concentration (µM)

Groups	A_1(Cig+VitC)	A_2(Cig)	B_1(Nic+VitC)	B_2(Nic)	C_1(Cont+VitC)	C_2(Control)
Mean nitrite conc (µM)	11.75±1.89**	21.00±1.29*	10.25±3.07	12.50±2.10	14.33±2.09	10.75±2.95

Values are ± SEM; n=5 in each group

**Significantly different from the positive control group (C_2) at 0.05*
*** Significantly different from the cigarette group (A_2) at 0.05*

Effect of Cigarette smoke and nicotine on Fasting Blood Glucose

Results show that neonates of rats exposed to CS had significantly lower BGL compared to the control (Table 2).The BGL of nicotine administered animals is not significantly different from the control

Groups	A_1(Cig+VitC)	A_2(Cig)	B_1(Nic+VitC)	B_2 (Nic)	C_1(Cont+VitC)	C_2(Control)
Blood glucose(mg/dL)	86.33±3.25**	61.00±2.03*	99.50±1.38	74.17±3.48	90.33±1.94	75.50±3.73

**Significantly different from the positive control group (C_2) at 0.05*
*** Significantly different from the cigarette group (A_2) at 0.05*

Discussion

It is still unclear whether the injurious effects of cigarette smoking in pregnancy are due to nicotine, and if so, what the effects are. Numerous other toxins in cigarette smoke adversely affect the placental circulation and/or fetal physiology and development (Jed *et al*, 2003).

The effects of CS on the NO pathways and Nitric Oxide Synthase (NOS) isoenzymes are controversial and may vary according to the disease, model or location of the NOS (Yates *et al*, 2001). For example, while exhaled NO has been shown to be decreased in humans after acute cigarette exposure, inducible nitric oxide synthase (iNOS) mRNA expression increased in the lungs of rats exposed to cigarette smoke, while neuronal nitric oxide synthase (nNOS) showed a longer term increase in both transcription and translation (Yates *et al*, 2001). CS has been shown, however, to cause a reduction in nitrite concentration and iNOS expression in a murine lung epithelial cell line in vitro (Kharitonov *et al*, 1995). In contrast, Comhair *et al* (2000) showed no change in iNOS expression in airway cells from healthy subjects exposed to CS. The effects of CS on NOS in the vasculature has shown a reduction in endothelial constitutive NO (ecNOS) in the pulmonary vessels in vitro and in vivo, genetic variation in man, while vascular intimal thickening and up-regulated iNOS has been described in mice (Comhair *et al*, 2000). These seemingly contradictory effects are probably explained in part by the different tissue situations and also by variation in the constituents of the CS (Comhair *et al*, 2000).

This work showed that the offspring of pregnant rats exposed to passive CS have significantly higher NO concentration than those not exposed in their prenatal life. This suggests that prenatal exposure to CS might activate various intracellular machinery in the offspring that promote eNOS or iNOS activity or promote expression of gene coding for these enzymes.

Increased NO production has been shown to promote respiratory infection following disease conditions. This might precipitate a vicious cycle of exacerbated and prolonged proatherogenic inflammatory responses, which would have potential to greatly accelerate an otherwise slow progression towards atherosclerosis and other disease conditions caused or potentiated by decreased endogenous NO production. Also this increased NO production may be immuno-suppressive.M.S Fikel *et al* (1992) reported that nitric oxide has negative inotropic effect on the heart. The depressor cardiovascular effect on brain stem function was also reported by Tseng *et al* (1996).

Prenatal exposure to nicotine administration on the other hand showed no significant difference in NO concentration when compared with the control group. Thus, the effect CS shown here could not be attributed to the pharmacological activity of nicotine, but may be related to the formation of smoking induced oxidative free radicals, as administration of anti-oxidants, vitamin C, reversed the effect. This agrees with the work of Seller and Bnait (1995) who reported detrimental effects of cigarette smoke in both high and low tar cigarettes.

This point is further buttressed by the fact that the group exposed to CS in the intrauterine life has a significantly higher NO concentration than those exposed to nicotine. Further studies need to be done to see if the effect of CS during intra uterine life on NO level will be blocked by anti- oxidants.

Previous research has demonstrated the ability of vitamin C administration, taken by nonsmokers two hours prior to being exposed to CS, to reduce the free radical damage, LDL oxidation associated with exposure to CS and the smoke-induced decline in total antioxidant defense. These beneficial effects were not observed in nonsmokers under normal conditions (Valkonen *et al*, 2000). Recently, researchers have shown that vitamin C may enhance endothelial function by promoting the synthesis of NO or by preventing its inactivation by scavenging superoxide radicals

(Valkonen, *et al*, 2000).The outcome of this research is consistent with these, as the NO concentration of the control group is found to increase following vitamin C administration.

Also, the NO concentration of the group exposed to CS and there after administered vitamin C was found to be significantly lower than those not administered vitamin C. There was no significant difference between the NO level of the nicotine and nicotine + vitamin C groups, suggesting that prenatal exposure to intramuscular nicotine injection (0.25mg kg/b.w) had no effect on formation of oxidative free radicals endogenous NO. Also, the NO concentration difference between cigarette + vitamin C group and the control group was not significant, indicating that vitamin C administration reversed this damaging effect of CS.

However, this work also showed that prenatal exposure to CS caused a significant reduction in the BGL when compared with the control group. Glucose is stored in muscle tissue as glycogen, thereby contributing to body weight. The low BGL in neonates of prenatally exposed rats might be a factor in low-birth weight observed in women who smoke during pregnancy. Moore and Persaud (2003) attributed the intra-uterine growth retardation and low birth weight infants of maternal smokers to the ability of nicotine to constrict uterine blood vessels, thereby reducing the amount of oxygen and nutrients available to the embryo from the maternal circulation.This impedes cell growth.

On the other hand, the difference between the BGL of those exposed to nicotine administration and the control group was not significant. Thus, the effect of prenatal exposure to CS on BGL could again not be attributed to the pharmacological activity of nicotine, but may be related to the formation of smoking induced oxidative free radicals.

Administration of vitamin C was shown to cause a significant increase the BGL of the control group. This increase in consistent in all the groups and are all statistically significant. Vitamin supplements thus increases BGL. This is consistent with the work of Donovan et al (2002) where administration of vitamin C to overnight fasted dogs resulted in significant elevation of the BGL and the elevated BGL were shown to be due to significant reduction in plasma insulin levels in the dogs, due mainly to impaired insulin release. A likely explanation for the impaired insulin release could be that competition between glucose and vitamin C for transport into the islet cells may have slowed glucose entry, thereby impairing the glucose-sensing apparatus of the islet cells (Donovan et al, 2002).

In conclusion, this study clearly demonstrate that prenatal exposure to passive smoke from cigarettes causes an increase in plasma NO and a decrease in BGL of offspring and these effects are not associated with the pharmacological activity of nicotine, because exposure to nicotine only did not significantly alter these variables. Also administration of the anti-oxidant vitamin C to the offspring after birth reverses the effects induced by prenatal exposure to CS.

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