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Changes in maternal and fetal nicotine distribution after maternal administration of monoclonal nicotine-specific antibody to rats

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Abstract

Vaccination against nicotine to elicit the production of nicotine-specific antibodies is a potential treatment for tobacco addiction which reduces nicotine distribution from serum to brain. Vaccination of pregnant rats also reduces the distribution of maternally-administered nicotine to fetal brain. Whether this is due to maternal antibody reducing the transfer of nicotine from mother to fetus, or to fetal antibody altering the distribution of nicotine within the fetus, is not clear. In the current study, passive immunization of rats with the murine monoclonal nicotine-specific antibody Nic311 was used as a surrogate for vaccination because antibody transfer to the fetus was anticipated to be lower than with vaccination. Pregnant rats received nicotine from gestational day (GD) 18–20 as frequent i.v. boluses to simulate nicotine exposure from smoking. Nic 311 was administered at doses of 30, 80 or 240 mg/kg on GD19. Fetal serum Nic311 levels on GD20 were <3% of concurrent maternal levels, but concentrations of up to 20 ug/ml in fetal serum were obtained owing to the very high levels in maternal serum. Accumulation of the chronically administered nicotine, measured on GD20, was not changed by Nic311 treatment in either maternal or fetal brain. The early distribution of nicotine to maternal brain, measured 5 min after a dose, was markedly reduced by Nic311, while the early distribution of nicotine to whole fetus and fetal brain was not substantially altered.. These data suggest that the limited transfer of Nic311 to the fetus in turn limits the ability of Nic311 to reduce nicotine distribution to fetal brain.

Keywords

Immunization; monoclonal antibody; nicotine; fetal; distribution

1. Introduction

Smoking during pregnancy is associated with adverse outcomes including prematurity, reduced birth weight, excess perinatal mortality, and sudden infant death syndrome [1]. Epidemiologic and case-control data also suggest associations of maternal smoking with developmental

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neurologic sequelae such as conduct disorder, attention deficit hyperactivity disorder, and a higher risk of becoming a smoker as a young adult [2–4]. Studies in rats strongly implicate nicotine as an important contributor to these adverse outcomes. Gestational exposure of rats to nicotine, at levels similar to smoking, is associated with long-lasting effects on brain gene expression [5], neurotransmitter homeostasis [6], the central control of respiration [7], and cognitive function [8]. The targeting of the central nervous system by these effects in rats and their persistence into adolescence are reminiscent of the effects of smoking during pregnancy, and consistent with a potential role for nicotine in mediating the harmful developmental effects of smoking during pregnancy.

Nicotine is the primary addictive component of tobacco [9]. Vaccination against nicotine is a potential treatment for tobacco addiction which focuses on the role of nicotine in smoking behavior [10]. Vaccination with a nicotine hapten-protein conjugate elicits the production of nicotine specific antibodies which bind nicotine in serum and extracellular fluid, reduce nicotine distribution to brain and attenuate behaviors relevant to nicotine addiction, including nicotine self-administration [11,12]. Several nicotine vaccines in phase II–III clinical trials have shown promise for facilitating smoking cessation [13]. Just as nicotine vaccines can alter nicotine distribution to tissues, they can alter nicotine transfer to the fetus. In rats, maternal vaccination prior to pregnancy reduced the distribution to fetal brain of a single nicotine dose administered to the mother on GD16–20 [14]. Because maternal nicotine-specific antibody is transferred to the fetus in rats, it is unclear whether reduced nicotine distribution to fetal brain was due to reduced transfer of nicotine from mother to fetus, or to altered nicotine distribution within the fetus due to the presence of nicotine-specific antibody in the fetus.

Understanding the effects of antibody transferred from mother to fetus on nicotine distribution is important because the extent of antibody transfer differs among species as well as stage of pregnancy. In rats following maternal vaccination, fetal serum levels of nicotine-specific antibody on GD20 (gestation period for rats 22 days) were 10% those of maternal serum [15]. In humans, fetal serum antibody levels increase during pregnancy and are equal to maternal levels at birth [16]. Extrapolating the effects of vaccination against nicotine from rats to humans therefore requires an understanding of the roles of maternal and fetal antibody in altering nicotine transfer to the fetus and to fetal brain. One approach to studying this question is passive maternal immunization using heterologous nicotine-specific antibodies that do not appreciably cross the placenta. In one such study maternally administered rabbit nicotine-specific IgG reduced the distribution to fetal brain of a single maternal nicotine dose to the same extent as vaccination [14]. However, the 30-fold difference in affinities of the rat and rabbit antibodies for nicotine made interpretation of these findings difficult. Passive immunization is also of interest because, like vaccination, it is effective in blocking many addictive effects of nicotine in rats and may represent an alternative to vaccination as a treatment for tobacco addiction [17–19]. Potential advantages of passive immunization over vaccination include control of the antibody dose and immediate onset of action.

In the current study, we used a murine monoclonal nicotine-specific antibody (Nic311) to study the effects of passive immunization on nicotine transfer from mother to fetus in rats. The K_d of Nic311 for nicotine (60 nM) is similar to that of serum from vaccinated rats (30–50 nM) so that comparisons of passive immunization with previous studies of vaccination are possible [20]. Nicotine was administered to the mother using a well characterized regimen which approximates the quantity and pattern of nicotine exposure from smoking [21].

2. Materials and Methods

2.1. Nicotine

(-)-Nicotine bitartrate (Sigma Chemical, St. Louis, MO) was diluted in phosphate buffered saline for administration to rats. ^3H -(-)-nicotine (PerkinElmer Life Sciences, Boston MA) was added to the final nicotine dose to allow differentiation of nicotine levels resulting from the final nicotine dose and accumulation of nicotine from the prior nicotine doses. All nicotine concentrations are expressed as weight of the base. Unlabeled nicotine concentrations were measured by gas chromatography [22]. ^3H -Nicotine concentrations were measured by scintillation counting and expressed as ng/ml nicotine by correcting for the specific activity of the administered ^3H -nicotine dose. Fetal serum and homogenates of whole fetus were decolorized prior to counting by adding 0.5 ml of 30% hydrogen peroxide to 1.0 ml of sample. Maternal brain nicotine concentrations were corrected for brain blood content [23]. Fetal brain nicotine concentrations were not similarly adjusted because values for fetal brain blood content are not available. However, the expected correction is small because brain blood content (in adult rats) is low (3.7%) and fetal blood and brain nicotine concentrations were similar.

2.2. Protein binding

The protein binding of unlabeled nicotine in serum was measured by equilibrium dialysis [24]. The unbound fraction of nicotine was calculated as the ratio of the buffer side to the serum side. The unbound concentration of nicotine in serum was calculated as total serum nicotine concentration $\times$ fraction unbound. Only maternal serum was analyzed for protein binding because the quantity of fetal serum available was insufficient.

2.3. Antibody

Nic311 is a previously characterized murine monoclonal IgG $_1$ kappa with a high affinity (K_d = 60 nM) and specificity for nicotine; cross reactivity <1% for the nicotine metabolites cotinine, nicotine-N-oxide and nornicotine, and the endogenous nicotinic receptor ligands acetylcholine and choline) [20]. Nic311 was purified by protein G chromatography. Nic311 concentrations were measured by ELISA using nicotine hapten-polyglutamate as the coating antigen [20]. Nic311 was diluted in phosphate buffered saline for administration to a volume of 1 ml for the lower doses and 2 ml for the highest dose. Control antibody was human nonspecific IgG (Sandoglobulin, Sandoz, Vienna, Austria) which has no effect on nicotine distribution in rats and is comparable in this regard to nonspecific murine monoclonal antibody [20].

2.4. Rats

Pregnant Holtzman Sprague Dawley rats were obtained on GD 3, singly housed, fed *ad lib*, and maintained on a 12h light/dark cycle. Indwelling cannulas were placed on GD18, 12 hours prior to the start of nicotine dosing, in the right jugular and left femoral veins using droperidol/fentanyl anesthesia, and externalized at the posterior upper back. A shoulder harness was placed and catheters were connected to the top of the cage through a tether and swivel that allowed free movement in the cage. Catheters were kept patent with heparinized saline prior to nicotine dosing.

2.5. Protocol

Group size for rats receiving Nic311 was limited to N=3 because of the expense of the higher doses of antibody. Control group size was N=5. These group sizes have been adequate in similar previous studies for demonstrating dose-related antibody or vaccine effects. Nicotine was administered via the jugular vein cannula starting at 10 p.m. on GD18 as i.v. bolus doses of 0.03 mg/kg delivered in a volume of 50 μl over 1 sec (equivalent on a mg/kg basis to the nicotine absorbed from smoking 2 cigarettes) every 14 minutes for 16 h/day, for a total daily dose of 2

mg/kg. This regimen has been previously characterized as providing both peak and mean, arterial and venous serum nicotine concentrations comparable to those of smokers [21]. The 16 h/day dosing schedule resembles typical patterns of smoking. Nic311 was administered on GD 19, 12 h after the start of nicotine dosing to allow nicotine levels to approach steady state and to simulate antibody use in a current smoker. Nic311 was infused over 10 min via the femoral catheter. Nicotine dosing was continued on GD20 until 10 a.m., the end of that day's nicotine dosing period and 40 h after the start of the protocol, when rats were anesthetized and the final nicotine dose, with ^3H -nicotine added, was delivered manually. Rats were sacrificed 5 minutes after the final nicotine dose by decapitation. Maternal trunk blood and brain were immediately collected, and serum and brain frozen until analyzed. Fetuses were removed, blood obtained by cardiac puncture and serum for an entire litter combined and frozen. Fetal brain was removed and frozen along with the remainder which was designated "whole fetus". Fetal samples from an entire litter were pooled for drug or antibody level analysis.

2.6. Statistical analysis

Maternal or fetal nicotine levels, antibody levels, protein binding parameters, and weights were compared by one-way ANOVA and Dunnett's post-tests. Because of the small number of animals per group, dose-response relationships for Nic311 effect on ^3H -nicotine distribution were also examined using linear trend analysis.

3. Results

3.1. Maternal and fetal weights

There were no significant differences among groups in maternal weight on GD18, number of fetuses per litter, or fetal weight (Table 1).

3.2. Antibody

Maternal and fetal serum Nic311 concentrations varied proportionately with the dose (Table 2). Fetal serum Nic311 concentrations were <3% of the corresponding maternal levels.

3.3. Maternal nicotine concentrations

Nic311 significantly and dose-dependently increased the retention of unlabeled (total) nicotine in maternal serum ($p < 0.01$, Figure 1, upper panel). The serum nicotine concentration in the 240 mg/kg group was 13.3 times that of controls. The maternal brain unlabeled nicotine concentration was not significantly altered by Nic311 treatment at any dose (figure 1, lower panel).

Nic311 also significantly increased the retention of ^3H -nicotine (representing the final dose) in maternal serum ($p < 0.01$, Figure 2, upper panel). In contrast to the absence of effect of Nic311 on unlabeled nicotine in brain, Nic311 dose-dependently reduced the ^3H -nicotine concentration in maternal brain ($p < 0.01$, Figure 2, lower panel). The maternal brain ^3H -nicotine concentration in the 240 mg/kg group was reduced by 58% compared to controls. Nic311 increased the percent of ^3H -nicotine bound in maternal serum ($p < 0.001$), and decreased the unbound concentration of ^3H -nicotine in serum ($p < 0.01$) in a dose-related manner (Table 3).

3.4. Fetal nicotine concentrations

Nic311 did not significantly alter the unlabeled nicotine concentration in fetal serum, brain or whole fetus (Figure 3), nor did it alter the concentrations of ^3H -nicotine in whole fetus or fetal serum (Figure 4). Nic311 did not significantly alter the fetal brain ^3H -nicotine concentration as measured by ANOVA ($p = 0.11$), but linear trend analysis showed a significant reduction

of brain ^3H -nicotine with increasing dose ($p = 0.02$). The fetal brain ^3H -nicotine concentration in the 240 mg/kg group was reduced by 39% compared to controls.

4. DISCUSSION

Because of the well established adverse effects of gestational nicotine exposure on fetal brain development, it is important to understand the influence of immunization against nicotine on the distribution of nicotine from mother to fetus and within the fetus. The principal findings of this study were that Nic311 did not alter the chronic accumulation of nicotine in the fetus or its early distribution (measured 5 minutes after a nicotine dose) to the whole fetus, and only minimally reduced the early distribution of nicotine to fetal brain. These data suggest that passive immunization with a monoclonal antibody such as Nic311 does not have a substantial protective effects in rats with regard to reducing nicotine distribution to either whole fetus or fetal brain.

Group size in this study was small because of the limited availability of Nic311. Nevertheless, clear and substantial dose-related effects of Nic311 were seen in maternal serum and brain nicotine levels, which is consistent with previous studies using similar sample sizes and doses. It is therefore unlikely that the small group size per se obscured an important effect of Nic311 on fetal nicotine levels.

The effects of Nic311 on *maternal* nicotine distribution were as anticipated; Nic311 did not alter the accumulation of nicotine in maternal brain, but did reduce its early distribution measured 5 minutes after a dose. Similar results have been obtained with vaccination against nicotine in adult male rats [25] or pregnant females [15], and with Nic311 administration in adult male rats [26]. The consistency of this finding in adult animals strongly supports the hypothesis that nicotine-specific antibodies serve primarily to slow nicotine distribution to brain rather than to prevent it. This time course is important because the rewarding effects of smoking are also greatest in the first few minutes after a cigarette [27]. The early effects of immunization on nicotine distribution to brain may therefore underlie its efficacy in attenuating the reinforcing effects of nicotine in rats [12]. The mechanisms by which immunization selectively reduces early nicotine distribution to brain is unclear.

Despite the decrease in unbound maternal nicotine concentration in serum in the presence of antibody, Nic311 did not reduce either the cumulative or the early distribution of nicotine to the whole fetus. Active transport of nicotine across the placenta could produce this result, and some evidence for active transport exists in a choriocarcinoma cell line [28], but the transfer of nicotine across the perfused ex-vivo placenta is similar to that of antipyrine and most readily explained by passive diffusion [29,30]. Possibly the small amount of Nic311 crossing the placenta was sufficient to bind and retain nicotine in the fetus, effectively opposing any effect of maternal Nic311 to decrease nicotine transfer to the fetus.

The early distribution of nicotine to fetal brain was not significantly reduced by Nic311 as measured by ANOVA. A trend toward reduced nicotine distribution to fetal brain with increasing Nic311 dose was suggested by linear trend analysis, despite a lack of effect of Nic311 on nicotine levels in the whole fetus. This suggests that Nic311 within the fetus acted as it does in the adult, to bind nicotine and reduce its distribution to fetal brain. This result has also been obtained in studies of vaccination against nicotine in rats [15]. With vaccination, nicotine-specific antibody transport into the fetus is greater than with Nic311; fetal serum nicotine-specific antibody levels with vaccination were 10% of maternal serum levels, while Nic311 serum levels in the current study were <3% of maternal serum levels. However, the Nic311 levels in maternal serum in the 240 mg/kg group was higher than nicotine-specific antibody levels after vaccination, so that even the small fraction of Nic311 transferred to the

fetus resulted in similar fetal antibody levels with Nic311 (20 ug/ml) as previously reported with vaccination (8–30 ug/ml). Passive immunization with Nic311, when administered at a very high dose, may therefore have acted in the same manner as vaccination, providing enough fetal Nic311 to alter nicotine distribution within the fetus.

Although vaccination and passive immunization with Nic311 both provide relatively low fetal antibody concentrations in rats, marked differences would be anticipated in humans. Serum antibody levels in humans at birth are approximately equal to maternal levels [16], much higher than the 10% of maternal levels reported for rats near term. Whether the transfer of larger amounts of nicotine-specific antibody to the fetus would increase or decrease fetal effects of nicotine is not entirely clear. If the results of the current study and previous rat studies with vaccination can be extrapolated to these higher fetal antibody levels, and nicotine-specific antibody within the fetus serves to protect the brain, then the early distribution of nicotine to fetal brain may also be reduced in humans after vaccination. Exposure of the whole fetus to nicotine might be greater as measured by total nicotine levels due to retention of nicotine in fetal serum and extracellular fluid by antibody, but most of this nicotine would be bound and the free, pharmacologically active fraction would be expected to be lower than in the absence of vaccination. Further study in species with greater transfer of nicotine to the fetus are needed to test this hypothesis.

The only previous study of passive immunization against nicotine during pregnancy utilized rabbit nicotine-specific antibodies with a substantially higher K_d (1.6 nM) than Nic311 (60 nM) and showed a reduction in nicotine distribution to both whole fetus and fetal brain. However, this study used only a single dose of nicotine. The higher affinity of this antiserum for nicotine could have contributed to the decrease in whole fetus nicotine levels, but this advantage would likely not have been maintained with chronic nicotine dosing because a previous study comparing Nic311 and this same rabbit antiserum in adult male rats showed no differences in effects on nicotine distribution between the antiserum and Nic311 during chronic nicotine dosing [26].

Despite widespread knowledge of the adverse effects of smoking during pregnancy, up to 25% of pregnant women continue to smoke throughout pregnancy [31]. There is considerable reluctance to use standard smoking cessation medications (nicotine replacement therapy, bupropion, nortriptyline) during pregnancy because of concerns about their potential adverse effects. Immunization against nicotine is appealing in this regard because the antibodies elicited are highly specific for nicotine and do not cross react with any as yet identified endogenous compounds or structures [11,26], and immunization against several infectious diseases is already used safely during pregnancy [32]. Whether maternal immunization prior to or during pregnancy would be safe for the fetus depends upon its net effects on nicotine distribution to the fetus. The current study of passive immunization, along with previous studies of vaccination, provides further evidence that immunization (at least in the rat model) does not increase the steady state exposure of the fetus to nicotine or the early peak exposure after a dose. These findings argue that immunization should not adversely affect these aspects of fetal exposure to maternally administered nicotine. Immunization also slows nicotine clearance and could prolong exposure to nicotine during abstinence, e.g. overnight in a smoker. Whether a slower decline in nicotine levels would adversely affect the fetus is not known, and further study including fetal outcomes would be helpful to address this question.

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Figure 1.

Maternal total (unlabeled) serum and brain nicotine concentrations measured 5 minutes after the final nicotine dose on GD20. Controls N=5, Nic311 groups N=3. Nicotine was administered as i.v. bolus doses of 0.03 mg/kg/d for 16 h/d on GD 19 and 20. Nic311 was administered as a single i.v. dose at the start of nicotine dosing on GD20. Rats were sacrificed 5 minutes after the final nicotine dose. ^3H -nicotine was added to the final nicotine dose to allow separate measurement of antibody effects on chronic nicotine accumulation (unlabeled nicotine) and its early distribution to brain following a dose (^3H -nicotine). Nic311 dose-dependently increased the maternal serum unlabeled nicotine concentration but did not alter the maternal brain unlabeled nicotine concentrations. Values are mean $\pm$ SD. ** $p < 0.01$

Figure 2.

Maternal ^3N -nicotine serum and brain concentrations measured 5 minutes after the final nicotine dose (containing ^3H -nicotine) was administered. Nic311 dose-dependently increased ^3N -nicotine retention in maternal serum. In contrast to its lack of effect on unlabeled brain nicotine, Nic311 significantly decreased the maternal brain ^3N -nicotine concentration.

** $p < 0.01$

Figure 3.
Fetal unlabeled nicotine concentrations in serum, brain and whole fetus were not significantly altered by Nic311 administration.

Figure 4.

Fetal ³H-nicotine concentrations in the whole fetus and fetal serum were not significantly altered by Nic311. The concentration of ³H-nicotine in fetal brain was significantly decreased as measured by linear trend analysis (# $p = 0.02$).

Table 1

Maternal and fetal weights, mean ± SD

Treatment		Maternal Weight g	Fetuses per Litter	Fetal Weight g/litter	Fetal Weight g/fetus
Control		442±23	15.8±1.9	61±9	3.9±0.4
Nic 311	30 mg/kg	443±12	13.0±3.0	48±18	3.6±0.7
	80 mg/kg	455±52	15.3±3.5	66±18	4.3±0.6
	240 mg/kg	425±33	13.7±1.5	51±6	3.7±0.5

No difference among groups in any of these parameters (p >0.30 for all comparisons).

Table 2

Nic311 serum concentrations, mean±SD

Treatment		Maternal Nic311 µg/ml	Fetal Nic311 µg/ml
Control		<0.001	<0.001
Nic 311	30 mg/kg	101±30	3±1
	80 mg/kg	270±50	6±5
	240 mg/kg	740±90	20±11

Table 3Protein binding in maternal serum of ^3H -nicotine, mean $\pm$ SD.

^3H -Nicotine ^a				
Treatment		Total Nicotine ng/ml	Bound Nicotine %	Unbound Nicotine ng/ml
Control		20 $\pm$ 2	3 $\pm$ 3	19 $\pm$ 2
Nic 311	30 mg/kg	47 $\pm$ 10 **	76 $\pm$ 2 **	11 $\pm$ 3 **
	80 mg/kg	106 $\pm$ 29 **	89 $\pm$ 3 **	12 $\pm$ 1 **
	240 mg/kg	153 $\pm$ 24 **	94 $\pm$ 1 **	9 $\pm$ 2 **

^aThe total nicotine concentration in serum was measured by gas chromatography, and the unbound concentration of nicotine in serum was calculated from the total nicotine concentration and the % bound measured by equilibrium dialysis.

**
p <0.01 compared to the Control group.