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Effects of nicotine-specific antibodies, Nic311 and Nic-IgG, on the transfer of nicotine across the human placenta

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Abstract

The adverse effects of smoking during pregnancy on fetal development are, in part, due to nicotine. These effects may be due to the actions of nicotine in fetal circulation or on placental functions. In pregnant rats, vaccination with a nicotine immunogen reduces the transfer of nicotine from the maternal to fetal circulation. However, extrapolation of these results to pregnant women might not be valid due to the well-recognized differences between human and rat placentas. In the current investigation, the effects of nicotine-specific antibodies on the transfer of nicotine from the maternal to fetal circuit of the dually perfused human placental lobule were determined. Two types of nicotine-specific antibodies were investigated; nicotine-specific mouse monoclonal antibody (Nic311, K_d for nicotine 60 nM) and IgG from rabbits vaccinated with a nicotine immunogen (Nic-IgG, K_d 1.6 nM). Transfer of the antibodies from maternal to fetal circuits was negligible. Both rabbit Nic-IgG and, to a lesser extent, mouse monoclonal Nic311 significantly reduced nicotine transfer from the maternal to fetal circuit as well as the retention of the drug by placental tissue. These effects were mediated by a substantial increase in the protein binding of nicotine and a reduction in the unbound nicotine concentration. Therefore, the data cited in this report suggest that the use of nicotine-specific antibodies might reduce fetal exposure to the drug, and that antibody affinity for nicotine is a key determinant of the extent of nicotine transfer.

Keywords: Nicotine; Nicotine-specific antibodies; Human placenta; Pregnancy; Transplacental transfer

1. Introduction

Smoking during pregnancy is a significant public health concern. Smoking of cigarettes by pregnant women is associated with an increase in the incidence of spontaneous abortion during the first trimester, an increase in the incidence of premature deliveries and perinatal mortality, and a decrease in the birth weight of newborns at term [1]. Heavy smoking by pregnant women may also result in neonatal withdrawal symptoms [2].

Nicotine is a principal alkaloid of tobacco leaves and is inhaled by cigarette smokers. Considerable data suggest that nicotine, as one of the components of cigarette smoke, may contribute to adverse pregnancy and birth outcomes. The effects of nicotine on fetal development can either be direct, depending on its concentration in the fetal circulation, and/or indirect due to its effects on placental functions. In rats, nicotine is a neuroteratogen that causes fetal brain cell damage with effects that persist postnatally [3]. Some of the effects of nicotine on the fetus are dose-dependent, suggesting that a reduction in fetal exposure to nicotine might decrease its adverse outcomes [4].

Advances in the behavioral and pharmacotherapy of smokers have been made but their impact is limited. The pharmacotherapy of nicotine users includes nicotine-replacement therapy with nicotine gum, inhaler, nasal spray or a patch as well as the use of the antidepressant bupropion. However, even with these pharmacotherapies most attempts to quit are unsuccessful [5]. Treatment of pregnant smokers has been a particular challenge because of concerns about mutual effects on the mother and developing fetus. A recent approach for treatment of drug dependence in general is the use of antibodies. Several

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vaccines for drugs of abuse such as cocaine, phencyclidine, and methamphetamine were developed [5,6]. The use of vaccines during pregnancy is of some interest because several vaccines directed against infectious diseases are already used during pregnancy without harming the fetus [7].

In animal models, the efficacy of immunotherapy against nicotine dependence has been demonstrated. Vaccination of adult rats elicits the production of nicotine-specific antibodies that bind and sequester nicotine in the serum and extra-cellular fluid, reducing its distribution to the brain and preventing several of the physiologic and behavioral effects of nicotine [8,9]. Moreover, Keyler et al. [10] demonstrated that vaccination of female rats before pregnancy, or passive immunization (administration of preformed antibody) during pregnancy can reduce exposure of the fetal brain to a single dose of maternally administered nicotine. Therefore, immunotherapy in the form of either vaccination or passive immunization with nicotine-specific antibodies has the potential of improving neonatal outcomes in humans by reducing fetal exposure to nicotine. However, extrapolation of the animal data to humans is not always valid due to differences in the anatomy and physiology of the human placenta from that of laboratory animals.

To the best of our knowledge, data on the safety and effects of vaccination and the use of nicotine-specific antibodies against nicotine, or other drugs of abuse, in pregnant women are not available. Ethical and safety concerns for the mother and fetus limit this type of in vivo investigations. On the other hand, the technique of in vitro dual perfusion of a placental lobule has proven a good predictor of in vivo conditions for the transfer of drugs from maternal to fetal circulation [11,12]. This technique has been successfully utilized by several investigators to determine transplacental transfer of therapeutic agents and, in particular, it has been utilized in our laboratory for the opiates buprenorphine, methadone, and the methadone congener L-acetylmethadol [13–15].

Therefore, the aim of this investigation is to determine the effects of two nicotine-specific antibodies namely, mouse monoclonal antibody (Nic311) and a higher affinity IgG from rabbits vaccinated with a nicotine immunogen (Nic-IgG), on the transfer of nicotine across term human placenta. The effects of nicotine and its antibodies on placental viability and functional parameters were also investigated.

2. Materials and methods

2.1. Chemicals

[^{3}H]-nicotine, at a specific activity of 86.7 Ci/mmol, was purchased from Perkin-Elmer Life Sciences, Inc. (Boston, MA) and [^{14}C]-antipyrine, at a specific activity of 4.7 mCi/mmol, as well as all other chemicals, were purchase from Sigma–Aldrich (St. Louis, MO).

2.2. Antibody source and properties

Monoclonal mouse nicotine-specific IgG (Nic311) was produced from a hybridoma cell line derived from mice immunized with NicVax (kindly provided by Nabi Biopharmaceuticals, Rockville, MD), which consists of the immunogen 3′-aminomethylnicotine linked to recombinant *Pseudomonas aeruginosa* exoprotein A [16]. The hybridoma was grown in protein-free medium and IgG was purified by Protein G chromatography [17]. Because monoclonal antibodies with a higher affinity for nicotine than Nic311 were not available, rabbit polyclonal nicotine-specific antiserum (Nic-IgG) was used instead to represent higher affinity binding of nicotine. Nic-IgG was produced using the same immunogen in rabbits (also provided by Nabi Biopharmaceuticals). Serum was purified by Protein G chromatography and contained both nicotine-specific and non-specific IgG, with the nicotine-specific content being 6.8%. Doses of this antibody were calculated and concentrations were reported as that for the nicotine-specific IgG content. Affinities of antibodies for nicotine were determined by radioimmunoassay and were 60 nM for Nic311 and 1.6 nM for rabbit antiserum [18]. Both antibodies are highly specific for nicotine, with $<3\%$ cross-reactivity to the nicotine metabolite cotinine and no cross-reactivity to a variety of other compounds including acetylcholine [16].

2.3. Placentas

Placentas were obtained immediately after vaginal or cesarean section delivery from the Labor and Delivery ward of John Sealy Hospital, University of Texas Medical Branch in Galveston according to a protocol approved by the Institutional Review Board. Placentas from uncomplicated term pregnancies from healthy non-smoking women were utilized in this study.

2.4. In vitro perfusion of human placental lobule

The technique of dual perfusion of a placental lobule was used according to the method of Miller et al. [11] and as described in detail in earlier reports from our laboratory [13]. Each placenta was visually examined for tears, an intact peripheral cotyledon selected, and the fetal artery and vein cannulated and perfused within 20 min of delivery. The flow rate of the medium in the fetal circuit was 2.8 mL/min. The perfused cotyledon was excised from the placenta, placed in the perfusion chamber, with the fetal side facing down, and supported by phosphate-buffered saline. The intervillous space on the maternal side was perfused by two catheters, piercing the basal plate, at a flow rate of 12 mL/min. The perfusion medium was made of

tissue culture medium M199 supplemented with the following: dextran (7.5 g/L in the maternal and 30 g/L in the fetal reservoir), 40 mg/L gentamicin sulfate, 80 mg/L sulfamethoxazole, and 16 mg/L trimethoprim. The maternal perfusate was gassed with 95% O_2, 5% CO_2, and the fetal perfusate was gassed with 95% N_2, 5% CO_2; both were maintained at 37 °C. Sodium bicarbonate was added to the maternal and fetal circuits to maintain the pH at 7.4 and 7.35, respectively. The maternal and fetal circuits were perfused with medium devoid of drugs for a period of 2 h (control period) to determine the baseline levels of the viability and functional parameters of the perfused lobule and to ensure its physical integrity. Perfusion of the placenta was terminated if one of the following events occurred: fetal arterial pressure exceeding 50 mmHg, a volume loss in the fetal circuit in excess of 2 mL/h, or a pO_2 difference between fetal vein and artery less than 60 mmHg.

The experimental period (4 h) followed the control period and was initiated by replacing the medium in the maternal and fetal reservoirs with a fresh one. The first group of placentas ($n = 9$) were transfused with nicotine only to determine the kinetics of its transfer from the maternal to fetal circuit. The concentration of nicotine transfused was 40 ng/mL and is based on that reported in the circulation of adult smokers [19]. The other two groups of placentas were co-transfused with nicotine and nicotine-specific mouse monoclonal antibody (Nic311; $n = 6$) or nicotine and IgG from rabbits vaccinated with a nicotine immunogen (Nic-IgG; $n = 3$). Nicotine and either antibody were added to the maternal reservoir at the beginning of each experiment. The concentration of Nic311 or Nic-IgG was 50 μg/mL and represented a 2.5:1 molar ratio of antibody binding sites to nicotine. This antibody concentration is within the range of serum antibody concentrations previously shown to attenuate the effects of nicotine in rats [16,20]. An inert, lipophilic, and highly diffusible marker compound antipyrine (AP) was co-transfused with the drug and its antibodies in each experiment to account for interplacental variations. The radioactive isotopes, [^{3}H]-nicotine and [^{14}C]-AP, 1.5 μCi of each, were added to the maternal reservoir and co-transfused to enhance their detection limits. Both the maternal and fetal circuits of the dually perfused placentas were re-circulated, i.e., a closed–closed system. The closed–closed system allows the determination of the amounts of nicotine and antibodies transferred to the fetal circuit as well as their distribution between the tissue, maternal, and fetal circuits. This system also allows accumulation of the metabolites formed, if any, during transfusion of the drug for 4 h.

The concentration of nicotine and AP was determined in 0.25 mL aliquots taken out from the maternal artery and fetal vein during the experimental period. To each aliquot 4 mL of ScintiSafe Econo 1 scintillation cocktail (Fisher Scientific International, Fair Lawn, NJ) was added and the radioactivity of the two isotopes determined simultaneously using a liquid scintillation analyzer (Perkin-Elmer Life Sciences). Albumin was not added to the perfusion medium because of its low binding to nicotine (4.9 ± 2.8%) [21]. Nicotine concentrations were also measured by gas chromatography [22]. Although gas chromatography is less sensitive, it allows measurement of the nicotine metabolite cotinine and was used to confirm that there was no appreciable metabolism of nicotine during the 4 h of perfusion. Reported nicotine concentrations are those measured by scintillation counting of radio-labeled nicotine. Drug concentrations measured in the presence of antibody represent total (bound plus unbound) nicotine. Aliquots of 0.25 mL were also collected at the same intervals of the experimental period from both circuits, centrifuged at 1000 × g for 5 min at 4 °C and the supernatant stored at −80 °C until the concentrations of Nic311 and Nic-IgG antibody were analyzed. Concentrations of Nic311 or nicotine-specific rabbit IgG in the medium or placenta were measured by ELISA as previously described [17]. Sensitivity of this assay is 10 ng/mL.

2.5. Binding of nicotine to dextran and antibodies in perfusion medium

The binding of [^{3}H]-nicotine to dextran was determined by gel filtration using Sephadex G-25 desalting columns (Amersham Pharmacia Biotech, Piscataway, NJ) as described previously [13] and was found to be negligible.

The effects of Nic311 and Nic-IgG on the protein binding of nicotine were determined in vitro by equilibrium dialysis using conditions intended to duplicate those at the start of ex vivo placental perfusion; [^{3}H]-nicotine 40 ng/mL, Nic311 or Nic-IgG 50 ug/mL. Because the nicotine-specific IgG content of Nic-IgG was 6.8%, the total protein concentration was 730 ug/mL. Control cells contained human non-specific IgG 730 ug/mL. Antibody was diluted into PBS, and dialyzed against phosphate-buffered saline pH 7.4 for 4 h [23]. The unbound nicotine concentration was calculated as the percent unbound multiplied by the starting nicotine concentration.

2.6. Assessment of placental viability parameters

The adverse effects of nicotine and/or nicotine-specific antibodies on the perfused tissue were determined utilizing specific viability and functional parameters [11]. During the initial 2 h of perfusion (control period), the baseline values for the following procedures were determined: oxygen delivery, consumption, and transfer (indicators of tissue viability) and the release of hCG (an indicator of tissue function). The same parameters were determined during the following experimental period of 4 h while nicotine and the antibodies were transfused. Samples were collected every 30 min from the fetal and maternal veins and arteries and immediately analyzed for pH, pO_2, and pCO_2 using a pH/blood gas analyzer (model 1620; Instrumentation Laboratory, Milan, Italy). Oxygen delivery rate,

consumption, and transfer were calculated according to the methods of Wier and Miller [24]. In addition, aliquots of 200 μL were taken from the maternal and fetal circuits during the control and experimental periods. They were centrifuged at 1000 × *g* for 5 min at 4 °C, and the supernatant was stored at −80 °C until analyzed. The concentration of hCG was determined using an IRMA kit (Diagnostics Products Corp., Los Angeles, CA).

Control placentas were perfused with medium only to determine the effect of time and experimental conditions on placental viability and functional parameters. The placentas were perfused for 120 min (control period), the medium changed, and the perfusion continued for another 240 min (experimental period).

2.7. Retention and accumulation of nicotine by the transfused placental lobule

The amount of nicotine present in the ''white'' part of the transfused tissue was determined in each experiment at the end of the experimental period of 4 h [13]. The tissue was dissected from the rest of the transfused lobule, weighed, and homogenized in saline. We added 1 mL of 1 M NaOH to an aliquot of the tissue homogenate of equal volume and incubated the samples overnight at 60 °C. Scintillation fluid (4 mL) was then added and the amount of radioactivity determined.

2.8. Statistical analysis

All values reported are expressed as mean ± S.D. The difference between the compared values was evaluated with the two-tailed *t*-test and considered significant when $P < 0.05$. A one-way repeated measures ANOVA was applied to calculate statistical significance in continuous measurements as in the effect of the drug on placental viability and functional parameters. Differences in protein binding were compared by one-way ANOVA with Tukey's post hoc comparison if the overall result was significant ($P < 0.05$).

3. Results

3.1. Placental viability and functional parameters

The transfusion of nicotine or its co-transfusion with either Nic311 or Nic-IgG did not affect oxygen delivery, transfer, consumption, or hCG release from the tissue. The values determined for the above parameters were within the range determined during the initial control period as well as in control placentas (Table 1). Therefore, nicotine and its antibodies, in the concentrations tested, did not adversely affect trophoblast tissue integrity or physiologic function.

3.2. Placental transfer of the marker compound antipyrine

The transfer of antipyrine (AP, 20 μg/mL), an inert and highly diffusible marker compound, from the maternal to fetal circuit was determined in each experiment to account for interplacental variations, i.e., it was co-transfused with nicotine and/or its antibodies. At the end of the transfusion period (4 h) the concentration of AP in the fetal vein was 10.25 ± 2.2 μg/mL or 51.3 ± 4.8% of its initial concentration (IC) in the maternal reservoir while its concentration in the maternal artery was 11.7 ± 1.8 μg/mL or 57.5 ± 2.0% of the IC. Therefore, AP is equally distributed between maternal and fetal circuits, and negligible amounts were retained by the tissue. More over, the transfer of AP was not affected by its co-transfusion with either nicotine, the antibodies (Nic311 and Nic-IgG), or the combination of nicotine and either antibody.

3.3. Transfer of antibody

The transfer of both Nic311 and Nic-IgG from maternal to fetal circuits was negligible throughout the entire 4 h, resulting in fetal/maternal concentration ratios at the end of the experiment of <1% (Table 2). Retention of antibody by the placenta was greater, with tissue/maternal circuit ratios of 0.14 ± 0.04 for Nic311 and 0.23 ± 0.05 for Nic-IgG.

Table 1
Effects of nicotine, nicotine–Nic311, and nicotine–Nic-IgG complexes on placental viability and functional parameters

Groups of placentas	hCG release (%)[a]	Oxygen delivery		Oxygen consumption		Oxygen transfer	
		Experimental period (mL/(min kg))	% of control period[b]	Experimental period (mL/(min kg))	% of control period[b]	Experimental period (mL/(min kg))	% of control period[b]
Control group[c]	87 ± 46	11 ± 2	83 ± 16	4 ± 1.6	103 ± 18	0.4 ± 0.1	81 ± 11
Experimental group 1 (nicotine only)	76 ± 12	14 ± 1	100 ± 27	3.8 ± 0.5	95 ± 46	0.9 ± 0.2	77 ± 24
Experimental group 2 (nicotine + Nic311)	70 ± 37	13 ± 0.6	103 ± 5	4.5 ± 0.6	76 ± 9	0.7 ± 0.1	78 ± 11
Experimental group 3 (nicotine + Nic-IgG)	70 ± 12	13 ± 1	92 ± 8	4.9 ± 0.6	95 ± 12	0.8 ± 0.2	84 ± 11

All values are expressed as mean ± S.D.

[a] The values obtained during the experimental period were expressed as a percentage of the respective values obtained during the control period of each perfusion, which were set as 100%.

[b] The tissue was perfused with medium only during the control period. The values obtained during the experimental period were expressed as a percentage of that obtained during the control period.

[c] No drug was added in the experimental period of the control group of placentas.

Table 2
Concentrations of Nic311 and Nic-IgG in the fetal circuit and placental tissue after transfusion

Tissue samples and length of transfusion period	Nicotine-specific antibody	
	Nic311	Nic-IgG
Fetal vein		
60 min	0.03 ± 0.02 μg/mL	0.017 ± 0 μg/mL
120 min	0.04 ± 0.01 μg/mL	0.04 ± 0.02 μg/mL
180 min	0.06 ± 0.01 μg/mL	0.06 ± 0.01 μg/mL
240 min	0.07 ± 0.01 μg/mL	0.06 ± 0.01 μg/mL
Placental tissue		
240 min	5.2 ± 1.6 μg/g	9 ± 2.1 μg/g

All values are expressed as mean ± S.D.

3.4. Transfer of nicotine

The transfer of nicotine (in the absence of antibody) from the maternal to fetal circuit during the experimental period of 4 h was determined in nine term placentas. The decline in the concentration of nicotine in the maternal artery was biphasic, rapid in the initial 30 min, then slower during the remaining 210 min (Fig. 1A). At the end of the initial 30 min, the concentration of nicotine in the maternal artery was 28.6 ± 3.0 ng/mL and represented approximately 70 ± 7% of its IC, i.e., 30% of it was transferred out of the maternal circuit and half of that occurred during the initial 5 min. At the end of the experiment (240 min), the concentration of nicotine in the maternal circuit was 15.4 ± 2.4 ng/mL and represented 38 ± 5% of its IC. In other words, approximately 60% of its IC was transferred out of the maternal circuit.

The rate of nicotine appearance in the fetal vein (Fig. 1B) coincided with its rapid decline in the maternal artery during the initial 5 min and its concentration in the fetal vein was 6.3 ± 3.6 ng/mL and represented 16 ± 9% of its IC, i.e., almost the same amount of nicotine transferred out of the maternal circuit during that period of time. At the end of 30 min, the period of fast decline in the concentration of nicotine in the maternal artery, the concentration of nicotine in the fetal vein reached its maximum of 12 ± 3.6 ng/mL or 33 ± 8% of its IC and did not change during the remaining experimental period of 210 min.

It should be noted here that the ratio of nicotine concentration in the fetal/maternal circuit at the end of the initial rapid phase of 30 min and at the end of the experiment (240 min) was 0.4 ± 0.1 and 0.8 ± 0.16 (Fig. 2), respectively. This suggests that placental tissue retained and accumulated nicotine during the last 210 min of its transfusion since the drug was not transferred to the fetal vein. It is interesting to note, that the transfer of nicotine to the fetal circuit at the end of 4 h was equal to approximately 84 ± 13% of the highly diffusible marker compound AP (Fig. 2), indicating that the diffusion rates of the two drugs are similar.

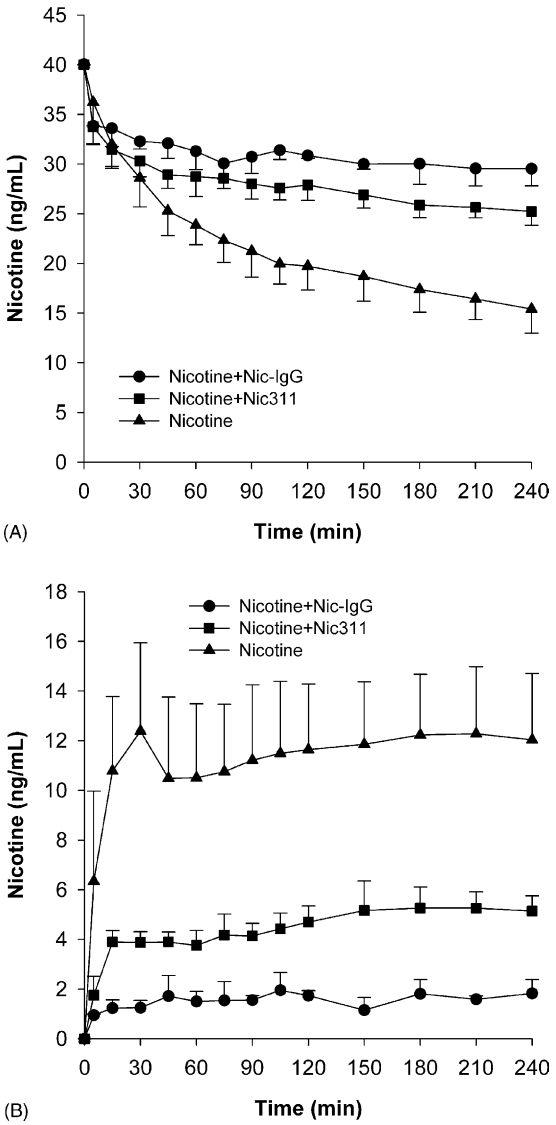

Fig. 1. (A) Concentration of nicotine in the maternal artery in the presence and absence of antibodies. The decline in the concentration of nicotine in the maternal artery in the presence of Nic311 and Nic-IgG was less pronounced ($P < 0.001$) than that of nicotine alone and represented 60 and 50%, respectively, of the nicotine concentration for the experimental group 1 (nicotine only). Data are expressed as mean ± S.D. (B) Concentration of nicotine in the fetal vein in the presence and absence of antibodies Nic-IgG and Nic311 significantly ($P < 0.001$) reduced the transfer of nicotine to the fetal circuit by 85 ± 12 and 56 ± 5%, respectively. Data are expressed as mean ± S.D.

3.5. Transfer of nicotine in the presence of nicotine-specific mouse monoclonal antibodies (Nic311)

In the maternal artery, the decline in the concentration of nicotine in the presence of Nic311 was also biphasic, i.e., similar to that observed for nicotine alone (Fig. 1A). It is also apparent that, during the initial rapid phase of 30 min, the presence of the antibodies neither affected the rate of the decline in the concentration of nicotine in the maternal artery nor its concentration at the end of that period when compared to nicotine alone. However, during the slower

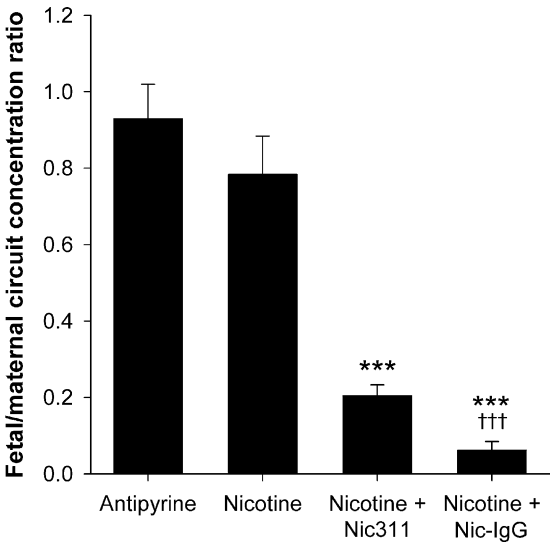

Fig. 2. The effect of the two nicotine-specific antibodies on the ratio of nicotine concentrations in the fetal/maternal circuit as compared with that of the marker compound antipyrine (AP). The ratio for the concentration of AP (the freely diffusible marker compound) in the fetal to maternal circuit is approximately 1. The ratio for nicotine concentrations (in the absence of antibodies) in the fetal to maternal circuit is 84 ± 17% that of AP, indicating that nicotine diffuses readily to the fetal circuit. The ratio for the concentration of nicotine in the fetal/maternal circuit in the presence of Nic311 and Nic-IgG was significantly lower ($P < 0.001$) than in their absence. This ratio represented 21 ± 2 and 7 ± 2%, respectively, of that for the ratio of AP concentration in the fetal/maternal circuits. Data are expressed as mean ± S.D. The symbol (***) indicates difference between the control and each of the experimental groups, i.e., in the absence and presence of antibodies; $P < 0.001$. The symbol (†††) indicates differences between the experimental groups ($P < 0.001$).

phase (30–240 min) the decline in the concentration of nicotine in the maternal artery was less pronounced in the presence of antibodies than in their absence. At the end of the experimental period of 240 min the concentration of nicotine, in the presence of Nic311 antibodies, amounted to 60% of that in their absence.

The transfer of nicotine to the fetal circuit in the presence of the antibody Nic311 resulted in a decrease in the rate and the amount of the drug transferred (Fig. 1B). At the end of the initial 5 min of nicotine transfusion in presence of Nic311, the concentration of nicotine in the fetal vein was 1.8 ± 0.8 ng/mL as compared to 6.4 ± 3.6 ng/mL in the absence of the antibodies. It is apparent that the presence of the antibodies decreased the amount of nicotine transferred to the fetal vein by 75% and the decrease was statistically significant ($P < 0.001$). It is also apparent (Fig. 1B) that the concentration of nicotine in the fetal vein does not reflect any significant changes between 30 and 240 min. However, during the latter period, the presence of Nic311 antibodies in the maternal artery resulted in decreasing the amount of nicotine transferred to the fetal vein by 58% of that transferred in absence of the antibodies.

The ratio of nicotine concentration in the fetal/maternal circuit in the presence of Nic311 at the end of the initial phase of 30 min was 0.13 ± 0.01, which is significantly ($P < 0.01$) lower than that observed for the same ratio in the absence of the antibodies (0.4 ± 0.1). At the end of 4 h of nicotine transfusion in the presence of Nic311, the ratio of the drug in the fetal/maternal circuit reached 0.2 ± 0.03 (Fig. 2). A comparison of nicotine transfer in the presence and absence of Nic311 to that of AP also illustrates the significant effect of the antibodies on nicotine transfer (Fig. 2).

3.6. Transfer of nicotine in the presence of IgG from rabbits vaccinated with nicotine immunogen

As observed for the transfer of nicotine in the presence and absence of monoclonal antibodies, the decline in the concentration of nicotine in the presence of Nic-IgG in the maternal artery was also biphasic (Fig. 1A), rapid in the initial 30 min of transfusion and slower, eventually leveling off, during the remaining period of 210 min. At the end of the experimental period of 240 min, the concentration of nicotine in the presence of Nic-IgG in the maternal artery was 32 ± 0.8 ng/mL, which is almost twice that for the concentration of nicotine (15.4 ± 2.4 ng/mL) when transfused in the absence of the antibodies and significantly higher ($P > 0.001$) than that for nicotine when transfused with its monoclonal antibodies (Nic311).

The amounts of nicotine transferred to the fetal circuit in the presence of Nic-IgG (Fig. 1B) were the lowest observed reaching 1.0 ± 0.04 and 1.8 ± 0.55 ng/mL after 5 and 240 min, respectively. It is interesting to note that the concentration of nicotine in the fetal circuit at the end of the experiment (240 min) was not significantly higher than that after 5 min.

Taken together, the above data indicate that when nicotine-specific antibodies, Nic311 and Nic-IgG, are added to nicotine in the maternal circuit, the addition will result in the formation of a drug–antibody complex that decreased the transfer of the drug to the fetal circuit. Furthermore, the effect observed for Nic-IgG on the transfer of nicotine, which was more pronounced than that of Nic311 ($P < 0.001$), is in agreement with the higher affinity of nicotine to Nic-IgG (K_d = 1.6 nM) than to Nic311 (K_d = 60 nM).

3.7. Nicotine retained by the tissue

The amounts of nicotine and its antibodies were determined in the transfused tissue at the end of the experimental period (Fig. 3 and Table 2, respectively). The concentration of nicotine in the tissue when transfused alone was 82 ± 42 ng/g and when co-transfused with either Nic311 or Nic-IgG was 20 ± 10 and 16.5 ± 2.0 ng/g, respectively. It is apparent that co-transfusion of either antibody with nicotine decreased the amount of the drug retained in the tissue by approximately 75% (Fig. 3). The amount of either antibody retained by the tissue was also negligible (Table 2). Taken together, these data support the

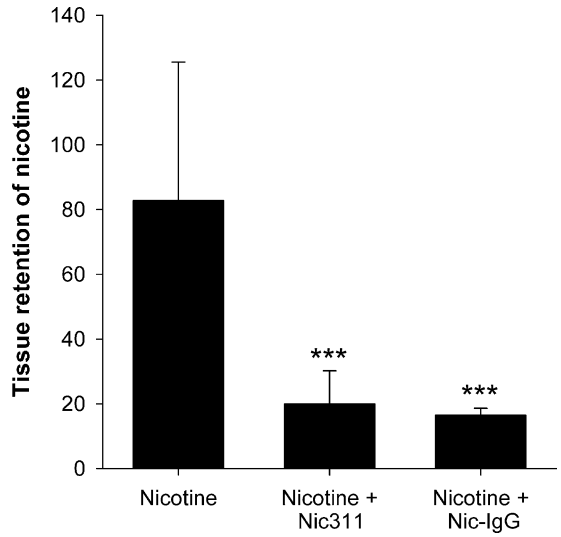

Fig. 3. The amounts of nicotine retained and accumulated by placental tissue in the presence and absence of antibodies. The amount of nicotine retained by placental tissue at the end of its transfusion for 4 h was 83 ± 43 ng/g. Transfusion of nicotine in the presence of either antibody (Nic311 or Nic-IgG) resulted in an approximately 75% decrease in the amount of the drug retained by the tissue. The symbol (***) indicates difference between the control and each of the experimental groups, i.e., in the absence and presence of antibodies; $P < 0.001$.

earlier conclusion, based on the transfer of nicotine to the fetal circuit in the presence and absence of the antibodies, that the formation of nicotine–antibody complex in the maternal reservoir decreased the transfer of the drug from the maternal circuit.

3.8. Protein binding

The binding of nicotine to control non-specific IgG was negligible. Binding of nicotine to Nic311 or Nic-IgG, at nicotine and protein concentrations simulating those of the perfusion medium in the ex vivo experiments, was substantially and significantly greater than that of control cells, and binding of nicotine to the higher affinity Nic-IgG was greater than its binding to Nic311 ($P < 0.001$ for all comparisons). The unbound nicotine concentration was reduced by both antibodies compared with control IgG and was reduced to a greater extent by Nic-IgG than Nic311 ($P < 0.001$ for all comparisons) (see Table 3 for more details).

Table 3
Protein binding of ^{3}H-nicotine determined in vitro by equilibrium dialysis using conditions intended to duplicate those at the start of ex vivo placental perfusion

Group	% of ^{3}H-nicotine bound	Unbound nicotine (ng/mL)
Control (non-specific IgG)	1.3 ± 0.6	37.3 ± 1.2
Nic311	86.7 ± 1.2	5.3 ± 0.6
Nic-IgG	98.3 ± 0.6	0.7 ± 0.2

$n = 3$; $P < 0.001$ for all comparisons.

4. Discussion

The hypothesis underlying this investigation is that antibodies specific to nicotine may decrease or prevent nicotine transfer across the human placenta and, consequently, nicotine concentration in the fetal circulation. This hypothesis was based on data obtained utilizing pregnant rats receiving a single dose of nicotine and immunized with rabbit nicotine-specific IgG that resulted in a 60% decrease in the concentration of nicotine in the fetal brain [10]. However, due to the well-recognized differences between rat and human placenta, data on the transplacental transfer of drugs could not be extrapolated from the first to the latter without approximation. Therefore, the aim of this investigation was to determine the effects of two nicotine-specific antibodies on the ex vivo transfer of nicotine across term human placenta. The model system utilized was that of dual perfusion of placental lobule.

The concentration of nicotine added to the maternal reservoir was 40 ng/mL, which is within the range reported in the circulation of adult male smokers [19]. Data reported here indicate that in the absence of antibodies, the concentration of nicotine in the fetal vein at the end of 4 h of transfusion represented approximately 30% of its initial concentration in the maternal artery. The high transfer of nicotine to the fetal circuit is in agreement with its properties of a low molecular weight (162 Da) compound. These data are also in agreement with information reported earlier by two groups investigating the transfer of nicotine utilizing the same model system but under slightly different experimental conditions. In one report, at the end of a 3-h experimental period, 38% of the nicotine transfused was transferred from the maternal to the fetal circuit [25]. In the second report, at the end of a 2-h experimental period and transfusing the same initial concentration of nicotine in the maternal artery as was used in this study, 40% of the drug was transferred to the fetal circuit [26].

The effect of two types of nicotine-specific antibodies on the transplacental transfer of nicotine was investigated; namely, mouse monoclonal antibody (Nic311) and IgG from rabbits vaccinated with a nicotine immunogen (Nic-IgG). These two antibodies were chosen to represent high (Nic-IgG, K_d 1.6 nM) and moderate (Nic311, K_d 60 nM) affinities for nicotine. The concentration of each antibody in the maternal reservoir was 50.0 μg/mL and was co-transfused with nicotine (40 ng/mL), thus achieving a molar ratio of antibody binding sites to nicotine of 2.5:1, which is similar to that measured in vivo in rats vaccinated with the nicotine immunogen and receiving nicotine at clinically relevant doses [9,27]. Moreover, data obtained by the equilibrium dialysis, using the concentration of nicotine and antibodies, utilized in this investigation revealed that the percentage of nicotine bound in the presence of Nic-IgG was higher than with Nic311 owing to the higher affinity of Nic-IgG to nicotine.

The addition of Nic311 to the maternal reservoir significantly reduced the transfer of nicotine to the fetal circuit. Moreover, the transfer of Nic311 to the fetal circuit was minimal (Table 2). Therefore, it is likely that the formation of the antibody–nicotine complex in the maternal circuit decreased the transfer of nicotine to the fetal circuit. These data are consistent with the significantly ($P < 0.001$) lower retention of nicotine by the tissue in the presence of Nic311 over that retained by the placenta in absence of the antibodies (Fig. 3).

The addition of Nic-IgG to the maternal reservoir and its co-transfusion with nicotine reduced its transfer to the fetal circuit by approximately 93% (Fig. 2). The more pronounced effect of Nic-IgG than that of Nic311 could be explained by the higher affinity of nicotine to Nic-IgG (1.6 nM) than to Nic311 (60 nM). These findings are consistent with a recent report that Nic-IgG is more effective than Nic311 in reducing the distribution of nicotine to the brain of adult male rats after administration of a single dose of nicotine [17].

Studies of immunization as a treatment for tobacco dependence have focused on vaccination rather than passive immunization because vaccination is simple, safe, inexpensive, and has a long-lasting effect. However, antibody concentrations in serum elicited by vaccination are highly variable, and 1–3 months are required to achieve these levels. Passive immunization would be more expensive than vaccination and might require more frequent dosing, but it has the advantage of controlling the antibody dose to assure an immediate onset and adequate effect. In addition, passive immunization may be more effective in protecting the fetus from exposure to nicotine, because it is not readily transferred to the fetal circulation. Homologous maternal IgG (e.g., from vaccination) is transferred to the fetus by placental FcRn receptors, which bind to the Fc region of IgG. In rats, passive immunization with rabbit Nic-IgG was more effective in reducing nicotine transfer to the fetus than vaccination, presumably because the greater transfer of nicotine-specific IgG to the fetus after vaccination, compared with the more limited transfer of passively infused rabbit IgG, provided a reservoir of binding capacity in the fetus that sequestered nicotine [10]. In humans, IgG transfer to the fetus mediated by FcRn receptors begins at the 17th week of gestation, and fetal IgG levels increase with age until they are equal to that of the mother at birth [28]. Because FcRn receptors are species-specific, it would be possible to use or design an antibody for passive immunization that does not cross the human placenta, maximizing its ability to protect the fetus from exposure to nicotine.

Finally, both nicotine and nicotine–antibody complex, at the concentrations investigated, did not have any adverse effects on the determined viability and functional parameters of placental tissue (Table 1). This is in agreement with the report indicating that only higher doses of nicotine (>1 mM), than the concentration used in the current study (0.25 μM) affect the contractile response of human umbilical, chorionic, and villus stem arteries and veins in the in vitro perfusion of isolated human placenta [29].

In summary, both rabbit Nic-IgG and, to a lesser extent, mouse monoclonal Nic311 significantly reduced nicotine transfer from the maternal to fetal circuit as well as its retention by placental tissue. Since nicotine is recognized as one of the components of tobacco responsible for adverse fetal outcomes, the data tested here suggest that immunization may be a potential mean for protection of the fetus from some of these adverse effects. If the data obtained in the model system utilized, dual perfusion of the placental lobule, is validated in vivo and under chronic nicotine dosing conditions, then treatment of the pregnant cigarette smoker with nicotine-specific antibodies might provide a strategy to reduce fetal nicotine exposure.

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