MONOCLONAL NICOTINE-SPECIFIC ANTIBODIES REDUCE NICOTINE DISTRIBUTION TO BRAIN IN RATS: DOSE- AND AFFINITY-RESPONSE RELATIONSHIPS

D. E. Keyler, S. A. Roiko, E. Benlhabib, M. G. LeSage, J. V. St. Peter, S. Stewart, S. Fuller, C. T. Le, and P. R. Pentel

Minneapolis Medical Research Foundation, Minneapolis, Minnesota (D.E.K., S.A.R., E.B., M.G.L., P.R.P.); University of Minnesota College of Pharmacy, Minneapolis, Minnesota (D.E.K., J.V.S.P.); University of Minnesota Medical School and Hennepin County Medical Center, Minneapolis, Minnesota (P.R.P.); Nabi Biopharmaceuticals, Rockville, Maryland (S.S., S.F.); and Division of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, Minnesota (C.T.L.)

Received February 18, 2005; accepted April 15, 2005

ABSTRACT:
Vaccination against nicotine is being studied as a potential treatment for nicotine dependence. Some of the limitations of vaccination, such as variability in antibody titer and affinity, might be overcome by instead using passive immunization with nicotine-specific monoclonal antibodies. The effects of antibodies on nicotine distribution to brain were studied using nicotine-specific monoclonal antibodies (NICmAbs) with Kd values ranging from 60 to 250 nM and a high-affinity polyclonal rabbit antisemur (Kd = 1.6 nM). Pretreatment with NICmAbs substantially increased the binding of nicotine in serum after a single nicotine dose, reduced the unbound nicotine concentration in serum, and reduced the distribution of nicotine to brain. Efficacy was directly related to antibody affinity for nicotine. Efficacy of the highest affinity NICmAb, NICmAb311, was dose-related, with the highest dose reducing nicotine distribution to brain by 78%. NICmAb311 decreased nicotine clearance by 90% and prolonged the terminal half-life of nicotine by 120%. At equivalent doses, NICmAb311 was less effective than the higher affinity rabbit antiserum but comparable efficacy could be achieved by increasing the NICmAb311 dose. These data suggest that passive immunization with nicotine-specific monoclonal antibodies substantially alters nicotine pharmacokinetics in a manner similar to that previously reported for vaccination against nicotine. Antibody efficacy is a function of both dose and affinity for nicotine.

Immunization has been studied as a potential treatment strategy for a variety of drug addictions, including heroin (Killian et al., 1978), cocaine (Carrera et al., 2002; Kantak et al., 2001), phencyclidine (Laurenzana et al., 2003), methamphetamine (McMillan et al., 2004), and nicotine (Pentel et al., 2000; Cerny et al., 2002; Lindblom et al., 2002; Sanderson et al., 2003; Carrera et al., 2004). Vaccines consisting of the drug linked to a foreign carrier protein elicit the production of drug-specific antibodies that bind drug in serum and extracellular fluid, reduce the unbound drug concentration, and reduce drug distribution to brain. Immunization has been shown to block or attenuate a variety of drug-induced behaviors in rats that are relevant to addiction, including locomotor activation, drug discrimination, and drug self-administration (for reviews, see Pentel and Malin, 2002; Haney and Kosten, 2004). These data suggest that immunization may have potential for the prevention or treatment of drug addiction. Clinical trials of vaccines for cocaine and nicotine addiction have been initiated (D. Hatsukami, personal communication; Kosten et al., 2002).

Two immunization strategies have been studied: active immunization, in which the experimental animal is vaccinated to elicit an immune response; and passive immunization, in which the experimental animal is administered exogenously produced drug-specific antibody (Pentel and Malin, 2002). Clinical interest has focused on vaccination because of its safety, prolonged effect, convenience (obviating the need for daily medication), and low cost. Passive immunization has been used primarily as an experimental expedient because of its immediate onset of effect compared to the 1 to 2 months required to develop a satisfactory antibody response to vaccination and because it allows control of the antibody dose (Pentel and Keyler, 2004). These same experimental advantages of passive immunization over vaccination could prove clinically useful as well. Rapid onset of effect could be advantageous for initiating treatment of patients in a timely manner, while they are motivated. Control of antibody dose could be important because individual responses to vaccination are variable (Kosten et al., 2002). Some patients may not achieve satisfactory antibody concentrations after vaccination, or others may require higher antibody concentrations than are achievable with vaccination. In addition, if a monoclonal antibody is used for passive immunization, an antibody with defined affinity and specificity can be selected for administration.

Nicotine addiction provides a useful model for studying passive immunization because both the pharmacokinetic and behavioral effects of vaccination have been well studied in rats. Vaccination reduces nicotine distribution to brain under a variety of clinically relevant acute and chronic nicotine-dosing conditions (Hieda et al., 2000; Pentel et al., 2000; Tuncok et al., 2001), reduces nicotine-

**ABBRVIATIONS:** NICmAb, monoclonal anti-nicotine antibody; mAb, monoclonal antibody; Nic-lgG, polyclonal rabbit anti-nicotine antiserum; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; AUC, area under the concentration-time curve; Clt, total clearance.
induced dopamine release from the nucleus accumbens (De Villiers et al., 2002), and attenuates the reinstatement of nicotine self-administration (Lindblom et al., 2002; LeSage et al., 2005). Passive immunization of rats with a nicotine-specific polyclonal rabbit antiserum, although less well studied, has similar effects on nicotine distribution to brain and attenuates nicotine discrimination (Malin et al., 2002) and the relief of nicotine abstinence by nicotine (Malin et al., 2001). In a recent study, passive immunization with a monoclonal antibody reduced nicotine-induced locomotor activation in a dose-related manner (Carrera et al., 2004). Although these data support the potential clinical use of passive immunization, the specific antibody characteristics that would provide optimal efficacy are unclear. Efforts with existing vaccines have focused on producing very high-affinity antibodies to maximize their ability to bind drug. However, the relationship of antibody affinity to efficacy is not established, and it is possible that antibodies with more modest affinities for nicotine would suffice. This is an important practical consideration because obtaining very high-affinity monoclonal antibodies to some antigens can be difficult. In addition, it is also possible that a very high-affinity antibody could result in greater antibody saturation with drug when large or repeated drug doses are administered and thus compromise efficacy (Keyler et al., 1999; Satoskar et al., 2003).

In the current study, a variety of monoclonal nicotine-specific monoclonal antibodies (NICmAbs) were evaluated to determine the relationship of antibody affinity to pharmacokinetic efficacy. Because it proved difficult to produce very high-affinity mAbs, a polyclonal nicotine-specific rabbit antiserum with higher affinity for nicotine was also studied. The most effective of the NICmAbs was further studied to determine the dose-response relationship for reducing nicotine distribution to brain and the effects of immunization on nicotine pharmacokinetic parameters.

Materials and Methods

**Drugs and Reagents.** (-)-Nicotine bitartrate and goat anti-IgG-peroxidase conjugate were obtained from Sigma-Aldrich (St. Louis, MO). Internal standards for nicotine assay were a gift from Dr. Peyton Jacob (University of California, San Francisco, CA). All nicotine doses and measured concentrations are expressed as the weight of the base.

**Production of Rabbit Nicotine-Specific IgG (Nic-IgG).** New Zealand White rabbits were immunized with trans-3'-aminoethyl nicotine conjugated to recombinant *Pseudomonas aeruginosa* exoprotein A in Freund’s adjuvant as described previously (Pentel et al., 2000). Immune rabbit serum was purified on a protein A-Sepharose 4 Fast Flow column (Amersham Biosciences Inc., Piscataway, NJ) equilibrated with PBS, eluted with 0.1 M glycine buffer at pH 9, and neutralized with 1 M Tris buffer at pH 9. The IgG was diaffiliated, concentrated, and brought to a concentration of 50 mg/ml total IgG in PBS.

The nicotine-specific IgG content of this fraction determined by ELISA was 5%. All doses of Nic-IgG administered to rats are expressed as milligrams of nicotine-specific IgG. This antiserum has been previously characterized as having low cross-reactivity with nicotine metabolites or acetylcholine (Pentel et al., 2000).

**Production of NICmAbs.** Mice were immunized with trans-3'-aminoethyl nicotine conjugated to recombinant *P. aeruginosa* exoprotein A adsorbed to alum, and hybridomas were prepared as described previously (Fuller et al., 1987). The fused cells were resuspended into a selection medium and seeded into 96-well tissue culture plates that contained a macrophage feeder layer (Stewart and Fuller, 1989). Supernatants of growing cultures were screened for nicotine-specific mAb secretors on a polyclutaminate-hapten conjugate used as antigen in ELISA assays (Stewart and Fuller, 1989). ELISA positives were rescreened on the nicotine metabolite cotinine to eliminate cotinine cross-reactive mAb secretors. Ascites produced by injecting established clones into mice were purified on a protein A column and further characterized. Affinity for nicotine was measured by radioimmunoassay (Muller, 1983).

**Control Antibodies.** Two control immunoglobulins were used in these experiments. The first experiment used control-mAb, a monoclonal IgG1 κ directed at the unrelated hapten desipramine. Because the availability of this monoclonal antibody was limited, polyclonal human IgG (control-IgG; Sandoglobulin; Sando, Vienna, Austria) was used for subsequent experiments (Pentel et al., 2000). Serum and brain nicotine concentrations are comparable in rats treated with either of these control antibodies and then administered nicotine (see control groups, Figs. 1 and 2).

**Characterization of Antibody.** Antibody affinity for nicotine was measured by radioimmunoassay (Muller, 1983). Nicotine-specific antibody concentrations in the serum of rats treated with NICmAbs were measured by ELISA calibrated using purified NICmAb (Keyler et al., 2005). Nicotine-specific antibody concentration in rabbit antisera was calculated from the binding capacity measured by radioimmunoassay (Muller, 1983). Antibody specificity was measured by competitive ELISA.

**Nicotine Assay.** Concentrations of nicotine and cotinine in serum or brain were measured by gas chromatography with nitrogen-phosphorus detection (Jacob et al., 1981; Hieda et al., 1997). Brain nicotine concentrations were corrected for brain blood content (Hieda et al., 1999).

**Effects of NICmAbs and Nic-IgG on Nicotine Distribution.** The purpose of this experiment was to compare the effects of antibodies with a range of affinities for nicotine (three NICmAbs and Nic-IgG) on nicotine distribution. Male Holtzman rats weighing 320 to 410 g and housed in pairs on a 12-h...
light/dark cycle were used for all experiments. Five groups of five to six rats were anesthetized, and femoral and jugular venous catheters were placed. Antibody pretreatment was administered via the femoral catheter. Controls received 10 mg/kg control-mAb, and the four antibody groups received 10 mg/kg NICmAb079 ($K_d = 250$ nM), NICmAb810 ($K_d = 70$ nM), NICmAb311 ($K_d = 60$ nM), or Nic-IgG ($K_d = 1.6$ nM) in 1 ml of PBS. Thirty minutes later, rats received 0.03 mg/kg nicotine over 10 s via the jugular catheter. Three minutes after nicotine dosing, rats were decapitated, and blood and brain were collected. Serum was separated for drug, antibody, and protein binding assay.

**Dose-Response for Effects of NICmAb311 on Nicotine Distribution.** The purpose of this experiment was to study the relationship between NICmAb dose and nicotine distribution. NICmAb311 was chosen to study because it had the lowest $K_d$ and the largest pharmacokinetic effect of the NICmAbs evaluated previously. Because the availability of NICmAb311 was limited, antibody administration was restricted to two rats per dose. Rats in this experiment were prepared and studied as described immediately above, receiving NICmAb311 at doses of 10 to 160 mg/kg, i.v., followed in 30 min by 0.03 mg/kg nicotine and decapitation and sampling 3 min after the nicotine dose.

**Nicotine Pharmacokinetic Parameters.** Groups of six rats were pretreated with 27 mg/kg either NICmAb311 or control-IgG. The 27 mg/kg dose was chosen to approximate the mean nicotine-specific antibody content of a rat vaccinated with the same immunogen (Pentel et al., 2000; Satoskar et al., 2003). This calculation was based upon a typical nicotine-specific serum antibody concentration of 0.2 mg/ml in previous studies of rats vaccinated with this immunogen (Pentel et al., 2000; Satoskar et al., 2003), and a steady-state volume of distribution of 0.125 l/kg for mouse IgG administered to rats (Bazin-Redureau et al., 1997). Antibody was administered i.p. in 0.5 ml of PBS. Twenty-four hours later, rats were anesthetized, and jugular and femoral venous catheters were placed. Nicotine (0.1 mg/kg) was administered via the jugular catheter over 10 s. Blood samples of increasing volume (0.5–1.5 ml) were collected from the femoral catheter at intervals of up to 4 h for controls and 20 h for rats treated with NICmAb311.

NICmAb311 was administered i.p. in this experiment, rather than i.v. as in the other experiments, as a convenience. To confirm that absorption of NICmAb311 via the i.p. route was satisfactory, six rats were anesthetized, and jugular and femoral venous catheters were placed. NICmAb311 (10 mg/kg) was administered to one group i.v. and to the other group i.p. Blood was sampled at 0.5, 1, 6, and 24 h for measurement of serum nicotine-specific antibody concentrations.

**Protein Binding.** Protein binding was measured by equilibrium dialysis of 0.9 ml of serum against PBS for 4 h at 37°C using Spectrapor 2 membranes (Spectrapor Labs, Rancho Dominguez, CA) as described previously (Pentel and Keyler, 1988).

**Estimation of Nicotine Pharmacokinetic Parameters.** Noncompartmental pharmacokinetic parameters were estimated from individual concentration-time data using WinNonlin version 4.1 (Pharsight, Mountain View, CA). $C_{max}$ was the highest measured value. The terminal elimination rate constant ($k_t$) was estimated by iterative least-squares regression using the terminal five to seven serum concentrations. Half-life was determined from the ratio $\ln2/k_t$. The area under the concentration-time curve (AUC) from time 0 to last observed concentration ($C_{last}$) was estimated using the log linear trapezoidal rule. The area under the curve (AUC) from $C_{last}$ to infinity was estimated as the ratio $C_{last}/k_t$. The AUC from time 0 to infinity was the sum of these area estimates. Total clearance ($Cl_t$) was estimated as the dose/AUC, and the steady-state volume of distribution as the product of clearance and area under the moment curve divided by AUC.

**Statistical Methods.** Correlations between antibody $K_d$ or antibody dose and various parameters (serum or brain nicotine concentration) were analyzed by linear regression, as were correlations between the unbound serum nicotine concentration and brain nicotine concentration. Differences between individual antibody-treated groups and controls were analyzed by one-way analysis of variance with Dunnett’s post hoc contrast if the overall test was significant at $p < 0.05$. Pharmacokinetic parameters were compared using two-sided $t$ tests.

**Results**

**Antibody Characteristics.** Antibody affinities for nicotine were NICmAb079 ($K_d = 250$ nM), NICmAb810 ($K_d = 70$ nM), NICmAb311 ($K_d = 60$ nM), and Nic-IgG ($K_d = 1.6$ nM). These antibodies provided a range of affinities varying by more than 2 orders of magnitude. NICmAb311 had the highest affinity of the available mAbs and was therefore focused upon in this study. Both NICmAb311 and Nic-IgG were highly specific for nicotine. NICmAb311 cross-reactivity was <1% for the nicotine metabolites (−)-cotinine, (±)-nicotine-N-oxide, and (±)-nornicotine, and <1% for the endogenous nicotinic receptor ligand acetylcholine. Nic-IgG cross-reactivity was 1.9% for (−)-cotinine, <1% for (±)-nicotine-N-oxide, 1.5% for (±)-nornicotine, and <1% for acetylcholine.

**Effects of Antibody Affinity on Nicotine Distribution.** All antibodies tested, except NICmAb079, significantly increased nicotine retention in serum, and all antibodies including NICmAb079 reduced nicotine distribution to brain compared with controls (Fig. 1). The magnitude of these effects was directly related to antibody affinity for nicotine, with a correlation of $r = 0.67$ ($p < 0.001$) between antibody $K_d$ and the serum nicotine concentration and $r = 0.69$ ($p < 0.001$) for the brain nicotine concentration. Nic-IgG ($K_d = 1.6$ nM) had the greatest effect, and NICmAb311 ($K_d = 60$ nM) had the greatest effect among mAbs. Protein binding showed similar affinity-related effects (Table 1), with the Nic-IgG group having the highest protein binding of nicotine ($r = 0.90$; $p < 0.001$) and the lowest unbound serum nicotine concentration ($r = 0.68$; $p < 0.001$). There was a close correlation between the unbound nicotine concentration in serum ($r = 0.99$; $p < 0.01$) and the brain nicotine concentration ($r = 0.99$; $p < 0.001$; Fig. 2, top).

Serum antibody concentrations were comparable 30 min after administration of the various NICmAbs (10 mg/kg) or Nic-IgG (230 ± 30, 230 ± 30, 230 ± 20, and 247 ± 31 μg/ml) for NICmAb079, NICmAb810, NICmAb311, and Nic-IgG, respectively ($p = N.S.$).

**Effects of NICmAb311 Dose on Nicotine Distribution.** There was a strong correlation between the log NICmAb311 dose and nicotine retention in serum ($r = 0.99$; $p < 0.001$) and nicotine distribution to brain ($r = 0.99$; $p < 0.01$) (Fig. 3). The highest NICmAb dose of 160
Effects of monoclonal nicotine-specific antibodies and Nic-IgG on nicotine protein binding in serum (mean ± S.D.)

The corresponding total serum and brain nicotine concentrations are shown in Fig. 1. There was a significant relationship between antibody $K_d$ and both the percentage of nicotine bound ($r = 0.99; p < 0.001$) and the unbound nicotine concentration in serum ($r = 0.68; p < 0.001$). Analysis of variance showed that all antibody groups differed from the control group.

<table>
<thead>
<tr>
<th>Treatments (n = 6)</th>
<th>$K_d$ (nM)</th>
<th>Nicotine Bound (%)</th>
<th>Unbound Nicotine (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-mAb</td>
<td>250</td>
<td>7 ± 2</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>NICmAb310</td>
<td>70</td>
<td>81 ± 7**</td>
<td>15 ± 4**</td>
</tr>
<tr>
<td>NICmAb311</td>
<td>60</td>
<td>95 ± 1**</td>
<td>3 ± 1**</td>
</tr>
<tr>
<td>Nic-IgG</td>
<td>1.6</td>
<td>90 ± 0.1**</td>
<td>3 ± 1**</td>
</tr>
</tbody>
</table>

* $p < 0.01$, ** $p < 0.01$ compared with control group.

Effects of NICmAb311 dose on nicotine binding in serum

Values are the mean ± S.D., except for unbound nicotine concentrations in the NICmAb groups, which show only the means because there were just two animals per group. Values for Nic-IgG, taken from the experiment illustrated in Table 1, are shown for comparison. Corresponding total serum and brain nicotine concentrations are shown in Fig. 3. There was a significant correlation between NICmAb311 dose and both the percentage of nicotine bound ($r = 0.93; p < 0.01$) and the unbound nicotine concentration in serum ($r = 0.98; p < 0.05$).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Nicotine Bound (%)</th>
<th>Unbound Nicotine (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-IgG</td>
<td>160</td>
<td>32 ± 8</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>NICmAb311</td>
<td>10</td>
<td>95.0</td>
<td>10.1</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>97.3</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>98.9</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>99.5</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>99.7</td>
<td>2.2</td>
</tr>
<tr>
<td>Nic-IgG</td>
<td>10</td>
<td>99.5 ± 0.1</td>
<td>2.7 ± 0.7</td>
</tr>
</tbody>
</table>

FIG. 3. Dose-response relationship for nicotine distribution after treatment with NICmAb311 compared with control-mAb. Data points for each of two rats at each dose are shown. Increasing NICmAb311 dose was associated with increasing serum nicotine concentration measured 3 min after a nicotine dose ($r = 0.99; p < 0.001$) and decreasing brain nicotine concentration ($r = 0.99; p < 0.01$). Values for rats pretreated with Nic-IgG, taken from the experiment described in Fig. 1, are shown for comparison.

mg/kg reduced brain nicotine concentration by 78% compared with control-IgG. The reduction in brain nicotine concentration produced by the 80 mg/kg NICmAb311 dose (71%) was similar to that of 10 mg/kg Nic-IgG determined in the previous experiment (69%). Serum protein binding of nicotine increased, and the unbound nicotine concentration decreased, with increasing NICmAb311 dose (Table 2; $p < 0.001$). There was a close correlation between the unbound nicotine concentration in serum and the brain nicotine concentration ($r = 0.99$; $p < 0.001$; Fig. 2, bottom). Serum NICmAb311 concentrations (mean of two values) measured 30 min after the antibody dose were dose-related; 0.22, 0.37, 0.93, 1.57, and 4.23 mg/ml with increasing NICmAb311 dose.

**Nicotine Pharmacokinetics (Fig. 4).** Pretreatment with NICmAb311 decreased nicotine $V_{d,ad}$ by 82% ($p < 0.01$), decreased $Cl_t$ by 90% ($p < 0.01$), and prolonged the terminal half-life by 120% ($p < 0.001$ compared with controls). Serum NICmAb311 concentrations were stable throughout the study period with values of 120 ± 33 µg/ml at 30 min, 100 ± 10 at 6 h, and 110 ± 030 µg/ml at 24 h after nicotine dosing.

**Route of NICmAb311 Dosing.** Serum antibody concentrations at 0.5, 1, 6, 24, and 48 h after antibody dosing were 195 ± 23, 188 ± 14, 133 ± 23, and 70 ± 10 µg/ml (i.v.) and 1 ± 1, 10 ± 7, 79 ± 36, and 70 ± 20 µg/ml i.p. Thus, serum antibody concentrations were comparable at 24 h, validating the use of the i.p. route for antibody administration in the preceding experiment, which examined nicotine pharmacokinetic parameters starting 24 h after i.p. administration of NICmAb311.

**Discussion**

These data show a clear relationship between monoclonal antibody affinity, dose, and efficacy for altering the distribution of a single dose of nicotine in the rat. Polyclonal nicotine-specific antiserum (Nic-IgG; $K_d = 1.6$ nM) was most effective, but a comparable effect was obtained with NICmAb311 ($K_d = 60$ nM) when administered at an 8-fold higher dose. NICmAb311 also markedly reduced nicotine clearance and prolonged its terminal half-life. These effects of passive immunization are strikingly similar to those reported previously after

FIG. 4. Serum nicotine concentrations (mean ± S.D.) after a single dose of nicotine 0.1 mg/kg in rats pretreated with NICmAb311 or control-IgG. Pharmacokinetic parameter estimates are shown in Table 3.
vaccination of rats against nicotine (Pentel et al., 2000; Satoskar et al., 2003). The data suggest that passive immunization with monoclonal antibodies could provide an alternative strategy to vaccination, and offer preliminary information regarding the $K_d$ and antibody dose required.

The NICmAbs used in this study represented a modest range of $K_d$ values for nicotine (60–250 nM). Because identification of higher affinity monoclonal antibodies has not been successful, polyclonal Nic-IgG was used to extend the range of $K_d$ values studied. It is possible that differences between the effects of Nic-IgG and the various NICmAbs were due to antibody characteristics other than $K_d$. We are aware of no data regarding the comparative pharmacokinetics of rabbit and mouse IgG in the rat. However, the measured serum nicotine-specific antibody concentrations in this study after either 3 mg of Nic-IgG or the various NICmAbs were similar. It is therefore likely that differences in efficacy between NIC-IgG and NICmAbs in the current study were predominantly due to their differences in $K_d$ for nicotine.

In the range of $K_d$ values evaluated, lower $K_d$ was associated with greater efficacy for retaining nicotine in serum, increasing protein binding of nicotine in serum, reducing unbound nicotine concentration in serum, and reducing nicotine distribution to brain. These data suggest that a monoclonal antibody with a lower $K_d$ than that of NICmAb311 would be even more effective than this monoclonal antibody. Nevertheless, several considerations suggest that NICmAb311 is sufficiently effective to be of interest as a means of reducing nicotine effects. First, the $K_d$ of 60 nM is only slightly higher than that of antibodies elicited by vaccination of rats (range 19–40 nM) with this same immunogen (Hieda et al., 2000; Pentel et al., 2000; K eyler et al., 2003), and vaccination has been shown to attenuate many nicotine-related effects in rats that are relevant to nicotine addiction (Lindblom et al., 2002; LeSage et al., 2005). In addition, the monoclonal nicotine-specific antibody studied by Carrera et al. (2004), with an even higher $K_d$ of 200 nM, produced a substantial reduction of the locomotor activating effect of a single nicotine dose (Carrera et al., 2004). Second, the magnitude of NICmAb311 effect is a function of dose. The 40 mg/kg dose of NICmAb311, which is within the range of antibody content of a vaccinated rat, produced a 20-fold increase in serum nicotine concentration and a 60% decrease in brain nicotine concentration compared with controls, values essentially identical to those reported after vaccination using the same protocol (Pentel et al., 2000). Higher NICmAb311 doses produced greater effects, and at the 80 mg/kg dose these effects were comparable with those of 3 mg of Nic-IgG. Thus, the lesser efficacy of NICmAb311 compared with Nic-IgG could be compensated with a suitable increase in NICmAb311 dose.

The feasibility of using passive immunization as a clinical therapy rests in part on the dose of antibody required. Even the highest NICmAb dose used in this study is well within the range of antibody doses used in other settings. Doses of methamphetamine- or phencyclidine-specific IgG of up to 1 g/kg (Hardin et al., 2002; McMillan et al., 2004), and desipramine-specific IgG doses of up to 5.2 g/kg (Pentel et al., 1991) have been administered to rats without adverse effect. Nonspecific IgG is administered to humans in doses of up to 2 g/kg for treatment of immunologic disorders (Oates-Whitehead et al., 2003) and is well tolerated. Even the highest NICmAb dose of 160 mg/kg used in this study is well within this range. The cost of such high antibody doses is an important consideration, but it is not clear that such high doses are required for efficacy. As noted above, the 40 mg/kg dose used in this study is similar to the estimated amount of antibody that is produced by vaccination and that is effective in reducing nicotine effects in rats, and it is also similar to the 50 mg/kg monoclonal nicotine-specific antibody dose recently reported to attenuate nicotine-induced locomotor activation in the rat (Carrera et al., 2004). Data are not yet available from ongoing clinical trials of vaccination to estimate effective serum antibody concentrations in humans.

The binding data confirm that NICmAb effects and mechanism of action are similar to those of vaccination against nicotine (Pentel and Malin, 2002). Antibodies are largely excluded from the brain by the blood-brain barrier owing to their large size, so that only unbound nicotine can distribute to brain (Satoskar et al., 2003). The highly significant correlations between unbound nicotine concentration in serum and brain nicotine concentration found in this study strongly support the hypothesis that immunization reduces nicotine distribution to brain by reducing the concentration of the unbound nicotine in serum.

This study examined nicotine distribution at 3 min after the nicotine dose. This time point is of interest because the rewarding effects of a cigarette are maximal in the first few minutes after a cigarette, and it is presumably these early behavioral effects that immunization will need to attenuate if it is to be of benefit in treating nicotine addiction (Henningfield et al., 1985). Previous studies with vaccination of rats against nicotine have shown similar effects on nicotine distribution as early as 30 s after the nicotine dose (Hieda et al., 1999) and persisting to at least 25 min (Satoskar et al., 2003). Although such studies need to be repeated with passive immunization, the close parallels between effects of vaccination reported previously and passive immunization found in the current study argue that passive immunization will be similarly effective.

The effects of NICmAb311 on nicotine pharmacokinetic parameters were also very similar to those reported previously after vaccination of rats against nicotine (K eyler et al., 1999). NICmAb311 markedly reduced nicotine clearance, decreased $V_{dss}$ and increased the terminal half-life. The increase in terminal half-life (from 1.1 h in controls to 2.5 h in rats pretreated with NICmAb311) contrasts with the much greater prolongation of phencyclidine’s terminal half-life to over 15 days in rats treated with phencyclidine-specific monoclonal antibody (Proksch et al., 2000). Although the reasons for this difference between drugs are not entirely clear, there were several substantial differences between the nicotine and phencyclidine protocols. The phencyclidine antibodies had a higher affinity for drug ($K_d = 1.8$ nM) (McClurkan et al., 1993) and were administered at a much higher dose (1 g/kg versus 27 mg/kg in the current study), both of which could
have led to enhanced drug binding and a more profound effect on drug elimination. In addition, phenycyclidine antibodies were administered at steady state produced by a continuous phenycyclidine infusion rather than the single nicotine dose used in the current study.

The slowing of nicotine elimination by nicotine-specific antibody presents the possibility that antibody could become saturated with nicotine after repeated or chronic dosing and become less effective in binding subsequently administered nicotine. However, any such saturation occurring after vaccination of rats does not seem to prevent vaccination from attenuating the effects of nicotine even under chronic dosing conditions that simulate cigarette smoking (Hieda et al., 2000). Since NlCnAb311 has a similar affinity for nicotine as does immune serum in vaccinated rats, a similar preservation of efficacy with chronic nicotine dosing would be expected with passive immunization, but this remains to be studied. It is also possible that the slower clearance and longer half-life of nicotine produced by NlCnAb311 could be beneficial. Population data suggest that smokers who have slower nicotine metabolism smoke less, perhaps because the effects of each cigarette last longer (Benowitz et al., 2002, Tynadle and Sellers, 2002). If so, it is possible that immunization could be used to facilitate smoking reduction. Further studies under chronic nicotine dosing conditions will be needed to evaluate this possibility and to comment further upon the optimal $K_d$ of antibodies used to modify the pharmacokinetic or behavioral effects of chronic nicotine.

Acknowledgments. We thank the National Cell Culture Center for assistance with production of NlCnAb311 and Matthew Dufek and T. J. Brannwell for technical support.

References


Address correspondence to: Dr. Paul Pentel, Department of Medicine, G5.205, Hennepin County Medical Center, 701 Park Ave. S., Minneapolis, MN 55415. E-pentel@umn.edu