

**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, P.R Pentel

Minneapolis, Medical Research Foundation, Minneapolis, MN 55415 (DEK, SAR, EB, MGL,
PRP) – primary lab for this study

University of Minnesota College of Pharmacy, Minneapolis, MN (DEK, JSP)

University of Minnesota Medical School and Hennepin County Medical Center Minneapolis,
MN (PRP)

Nabi Biopharmaceuticals, Rockville, MD (SS, SF)

Division of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, MN
(CTL)

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Corresponding author:

Paul Pentel, MD

Dept. Medicine, G5.205

Hennepin County Medical Center

701 Park Ave S.

Minneapolis, MN 55415

Phone 612-873-6426

Fax 612-904-4299

email pentel@umn.edu

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Abbreviations: NICmAb - monoclonal anti-nicotine antibodies; Nic-IgG - polyclonal rabbit anti-nicotine antiserum; rEPA - recombinant *Pseudomonas* exoprotein A

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 Kds ranging from 60 to 250 nM and a high affinity polyclonal rabbit antiserum (Kd 1.6 nM). Pretreatment with NICmAb substantially increased the binding of nicotine in serum following 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., 2001; 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 which 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 (reviewed in (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 (personal communication, D. Hatsukami, and (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 animals 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 1-2 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-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., In press). 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). While these data support the potential clinical use of passive immunization, the specific antibody characteristics which would provide optimal efficacy are unclear. Efforts with existing vaccines have focused on producing very high affinity antibodies in order 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 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.

METHODS

Drugs and reagents. (-)-Nicotine bitartrate and goat anti-IgG-peroxidase conjugate were obtained from Sigma Chemical Co. (St. Louis, MO). Internal standards for nicotine assay were a gift from Dr. Peyton Jacob. 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'-aminomethyl nicotine conjugated to recombinant *Pseudomonas aeruginosa* exoprotein A in Freund's adjuvant as previously described (Pentel et al., 2000). Immune rabbit serum was purified on a Protein G Sepharose 4 Fast Flow column (Pharmacia, Piscataway, NJ) equilibrated with PBS, eluted with 0.1 M glycine buffer at pH 2.7, and neutralized with 1 M Tris buffer at pH 9. The IgG was diafiltered, 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 mg 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 nicotine-specific monoclonal antibodies (NICmAb). Mice were immunized with *trans*-3'-aminomethyl nicotine conjugated to recombinant *pseudomonas aeruginosa* exoprotein A adsorbed to alum and hybridomas were prepared as previously described (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 [24]. Supernatants of growing cultures were screened for nicotine-specific mAb secretors on a polyglutamate-hapten conjugate

used as antigen in ELISA assays (Stewart and Fuller, 1989). ELISA positives were re-screened 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 kappa directed at the unrelated hapten desipramine. Because the availability of this monoclonal antibody was limited, polyclonal human IgG (Control-IgG; Sandoglobulin, Sandoz, 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, Figures 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., In press). Nicotine-specific antibody concentration in rabbit antiserum 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 5-6 rats were anesthetized and femoral and jugular venous catheters placed. Antibody pretreatment was administered via the femoral catheter. Controls received Control-mAb 10 mg/kg, and the 4 antibody groups received 10 mg/kg of NICmAb079 (Kd 250 nM), NICmAb810 (Kd 70 nM), NICmAb311 (Kd 60 nM) or Nic-IgG (Kd 1.6 nM) in 1 ml PBS. Thirty minutes later, rats received 0.03 mg/kg nicotine over 10 sec via the jugular catheter. Three minutes after nicotine dosing, rats were decapitated and blood and brain 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 Kd and the largest pharmacokinetic effect of the NICmAbs previously evaluated. Because the availability of NICmAb311 was limited, antibody administration was restricted to 2 rats per dose. Rats in this experiment were prepared and studied as described immediately above, receiving NICmAb311 at doses of 10-160 mg/kg i.v. followed in 30 minutes by nicotine 0.03 mg/kg and decapitation and sampling 3 minutes after the nicotine dose.

Nicotine pharmacokinetic parameters. Groups of six rats were pretreated with 27 mg/kg of 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 PBS. Twenty four hours later, rats were anesthetized and jugular and femoral venous catheters placed. Nicotine 0.1 mg/kg was administered via the jugular catheter over 10 sec. Blood samples of increasing volume (0.5 to 1.5 ml) were collected from the femoral catheter at intervals of up to 4h 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 in this study, as a convenience. To confirm that absorption of NICmAb311 via the i.p. route was satisfactory, 6 rats were anesthetized and jugular and femoral venous catheters 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 serum against PBS for 4h at 37°C using Spectrapor 2 membranes (Spectrapor Labs, Rancho Dominguez, CA) as previously described (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) was estimated by iterative least squares regression using the

terminal 5 to 7 serum concentrations. Half-life was determined from the ratio $\ln 2/k$. 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 terminal area from C_{last} to infinity was estimated as the ratio C_{last}/k . 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 Kd 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 ANOVA 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 (Kd 250 nM), NICmAb810 (Kd 70 nM), NICmAb311 (Kd 60 nM) and Nic-IgG (Kd 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, (\pm)-nicotine-N-oxide and (\pm)-nornicotine, and <1% for the endogenous nicotinic receptor ligand acetylcholine. Nic-IgG cross reactivity was (-)-cotinine 1.9%, (\pm)-nicotine-N-oxide <1%, (\pm)-nornicotine 1.5% , and acetylcholine <1%.

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 to controls (Figure 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 Kd and the serum nicotine concentration and $r = 0.69$ ($p < 0.001$) for the brain nicotine concentration. Nic-IgG (Kd =1.6 nM) had the greatest effect, and NICmAb311 (Kd = 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 ($r = 0.99$, $p < 0.001$, Figure 2, top).

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

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 ($r = 0.99$, $p < 0.01$) (Figure 3). The highest NICmAb dose of 160 mg/kg reduced brain nicotine concentration by 78% compared to 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 ($r = 0.99$, $p < 0.001$) (Figure 2, bottom). Serum NICmAb311 concentrations (mean of 2 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 (figure 4). Pretreatment with NICmAb311 decreased nicotine V_{dss} by 82% ($p < 0.01$), decreased Cl by 90% ($p < 0.01$), and prolonged the terminal half-life by 120% ($p < 0.001$ compared to controls). Serum NICmAb311 concentrations were stable throughout the study period with values of 120 ± 33 $\mu\text{g/ml}$ at 30 min 100 ± 10 at 6 h, and 110 ± 30 $\mu\text{g/ml}$ 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 $\mu\text{g/ml}$ (i.v.) and 1 ± 1 , 10 ± 7 , 79 ± 36 , and 70 ± 20 $\mu\text{g/ml}$ (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, Kd 1.6 nM) was most effective, but a comparable effect was obtained with NICmAb311 (Kd 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 previously reported after 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 Kd and antibody dose required.

The NICmAbs used in this study represented a modest range of Kds for nicotine (60 to 250 nM). Because identification of higher affinity monoclonal antibodies has not been successful, polyclonal Nic-IgG was used to extend the range of Kds studied. It is possible that differences between the effects of Nic-IgG and the various NICmAbs were due to antibody characteristics other than Kd. 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 Kd for nicotine.

In the range of Kds evaluated, lower Kd was associated with greater efficacy for retaining nicotine in serum, increasing protein binding of nicotine in serum, reducing unbound nicotine

concentration in serum, and reduced 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 to 40 nM) with this same immunogen (Hieda et al., 2000; Pentel et al., 2000; Keyler 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., In press). In addition, the monoclonal nicotine-specific antibody studied by Carrera et al, 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 concentration compared to 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 to those of 3 mg Nic-IgG. Thus the lesser efficacy of NICmAb311 compared to 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 which is produced by vaccination and which is effective in reducing nicotine effects in rats, and 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 minutes 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 sec after the nicotine dose [29] and persisting to at least 25 minutes [22]. While 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 quite similar to those reported previously after vaccination of rats against nicotine (Keyler 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). While the reasons for this difference between drugs is 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 v. 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, phencyclidine antibodies were administered at steady state produced by a continuous phencyclidine 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 appear to prevent vaccination from attenuating the effects of nicotine even under chronic dosing conditions which simulate cigarette smoking

(Hieda et al., 2000). Since NICmAb311 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 remains to be studied. It is also possible that the slower clearance and longer half-life of nicotine produced by NICmAb311 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; Tyndale 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 comment further upon the optimal K_d of antibodies used to modify the pharmacokinetic or behavioral effects of chronic nicotine.

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REFERENCES

- Bazin-Redureau MI, Renard CB and Scherrmann JG (1997) Pharmacokinetics of heterologous and homologous immunoglobulin G, F(ab')₂ and Fab after intravenous administration in the rat. *Journal of Pharmacy and Pharmacology* **49**:277-281.
- Benowitz NL, Perez-Stable EJ, Herrera B and Jacob P, 3rd (2002) Slower metabolism and reduced intake of nicotine from cigarette smoking in Chinese-Americans. *J Natl Cancer Inst* **94**:108-115.
- Carrera MR, Ashley JA, Hoffman TZ, Isomura S, Wirsching P, Koob GF and Janda KD (2004) Investigations using immunization to attenuate the psychoactive effects of nicotine. *Bioorg Med Chem* **12**:563-570.
- Carrera MR, Ashley JA, Wirsching P, Koob GF and Janda KD (2001) A second-generation vaccine protects against the psychoactive effects of cocaine. *Proc Natl Acad Sci U S A* **98**:1988-1992.
- Cerny EH, Levy R, Mauel J, Mpandi M, Mutter M, Henzelin-Nkubana C, Patiny L, Tuchscherer G and Cerny T (2002) Preclinical development of a vaccine 'Against Smoking'. *Onkologie* **25**:406-411.
- De Villiers SH, Lindblom N, Kalayanov G, Gordon S, Malmerfelt A, Johansson AM and Svensson TH (2002) Active Immunization against Nicotine Suppresses Nicotine-Induced Dopamine Release in the Rat Nucleus accumbens Shell. *Respiration* **69**:247-253.
- Fuller S, Takahashi M and Hurrell JGR, Direct ELISA screening of serum and hybridoma supernatants. In: F.M. Ausubel et al. (Eds.), *Current Protocols in Molecular Biology*. Green Publishing Associates and Wiley Inter-science, New York, Chapter 11 (1987)
- Direct ELISA screening of serum and hybridoma supernatants, in *Current Protocols in*

Molecular Biology (ed AFMe), Green Publishing Associates and Wiley Inter-science, New York.

Haney M and Kosten TR (2004) Therapeutic vaccines for substance dependence. *Expert Rev Vaccines* **3**:11-18.

Hardin JS, Wessinger WD, Wenger GR, Proksch JW, Laurenzana EM and Owens SM (2002) A single dose of monoclonal anti-phencyclidine IgG offers long-term reductions in phencyclidine behavioral effects in rats. *J Pharmacol Exp Ther* **302**:119-126.

Henningfield JE, Miyasato K and Jasinski DR (1985) Abuse liability and pharmacodynamic characteristics of intravenous and inhaled nicotine. *J Pharmacol Exp Ther* **234**:1-12.

Hieda Y, Keyler DE, Ennifar S, Fattom A and Pentel PR (2000) Vaccination against nicotine during continued nicotine administration in rats: immunogenicity of the vaccine and effects on nicotine distribution to brain. *Int J Immunopharmacol* **22**:809-819.

Hieda Y, Keyler DE, Vandevort JT, Kane JK, Ross CA, Raphael DE, Niedbalas RS and Pentel PR (1997) Active immunization alters the plasma nicotine concentration in rats. *J Pharmacol Exp Ther* **283**:1076-1081.

Hieda Y, Keyler DE, VanDeVoort JT, Niedbala RS, Raphael DE, Ross CA and Pentel PR (1999) Immunization of rats reduces nicotine distribution to brain. *Psychopharmacology* **143**:150-157.

Jacob P, Wilson M and Benowitz NL (1981) Improved gas chromatographic method for the determination of nicotine and cotinine in biologic fluids. *J Chromatog* **222**:61-70.

Kantak KM, Collins SL, Bond J and Fox BS (2001) Time course of changes in cocaine self-administration behavior in rats during immunization with the cocaine vaccine IPC-1010. *Psychopharmacol* **153**:334-340.

Keyler DE, Dufek MB, Calvin AD, Bramwell TJ, LeSage MG, Raphael DE, Ross CA, Le CT and Pentel PR (In press) Reduced nicotine distribution from mother to fetal brain in rats vaccinated against nicotine: Time course and influence of nicotine dosing regimen.

Biochemical Pharmacology.

Keyler DE, Hieda Y, St.Peter J and Pentel PR (1999) Altered disposition of repeated nicotine doses in rats immunized against nicotine. *Nicotine and Tobacco Research* **1**:241-249.

Keyler DE, Shoeman D, LeSage MG, Calvin AD and Pentel PR (2003) Maternal vaccination against nicotine reduces nicotine distribution to fetal brain in rats. *J Pharmacol Exp Ther* **305**:587-592.

Killian A, Bonese K, Rothberg RM, Wainer BH and Schuster CR (1978) Effects of passive immunization against morphine on heroin self-administration. *Pharmacol Biochem Behav* **9**:347-352.

Kosten TR, Gonsai K, St Clair Roberts J, Jack L, Bond J, Mitchell E and Fox B (2002) Phase II human study of cocaine vaccine TA-CD. *CPDD Annual Meeting, Quebec City*.

Laurenzana EM, Gunnell MG, Gentry WB and Owens SM (2003) Treatment of adverse effects of excessive phencyclidine exposure in rats with a minimal dose of monoclonal antibody. *J Pharmacol Exp Ther* **306**:1092-1098.

LeSage MG, Keyler DE, Hieda Y, Collins G, Burroughs D, Le CE and Pentel PR (In press) Effects of nicotine conjugate vaccine on the acquisition and maintenance of nicotine self-administration in rats. *Psychopharm*.

Lindblom N, De Villiers SH, Kalayanov G, Gordon S, Johansson AM and Svensson TH (2002) Active Immunization against Nicotine Prevents Reinstatement of Nicotine-Seeking Behavior in Rats. *Respiration* **69**:254-260.

- Malin DH, Alvarado CL, Woodhouse KS, Karp H, Urdiales H, Lay D, Appleby P, Moon WD, Ennifar S, Basham LE and Fattom A (2002) Passive immunization against nicotine attenuates nicotine discrimination. *Life Sciences* **70**:2793-2798.
- Malin DH, Lake JR, Lin A, Saldana M, Balch L, Irvin ML, Chandrasekara H, Alvarado CL, Hieda Y, Keyler DE, Pentel PR, Ennifar S, Basham LE, Naso R and Fattom A (2001) Passive immunization against nicotine prevents nicotine alleviation of nicotine abstinence syndrome. *Pharmacol Biochem Behav* **68**:87-92.
- McClurkan MB, Valentine JL, Arnold L and Owens SM (1993) Disposition of a monoclonal anti-phencyclidine Fab fragment of immunoglobulin G in rats. *J Pharmacol Exp Ther* **266**:1439-1445.
- McMillan DE, Hardwick WC, Li M, Gunnell MG, Carroll FI, Abraham P and Owens SM (2004) Effects of murine-derived anti-methamphetamine monoclonal antibodies on (+)-methamphetamine self-administration in the rat. *J Pharmacol Exp Ther* **309**:1248-1255.
- Muller R (1983) Determination of affinity and specificity of anti-hapten antibodies by competitive radioimmunoassay. *Methods of Enzymology* **92**:589-601.
- Oates-Whitehead RM, Baumer JH, Haines L, Love S, Maconochie IK, Gupta A, Roman K, Dua JS and Flynn I (2003) Intravenous immunoglobulin for the treatment of Kawasaki disease in children. *Cochrane Database Syst Rev*:CD004000.
- Pentel PR, Brunn GJ, Pond SM and Keyler DE (1991) Pretreatment with drug-specific antibody reduces desipramine cardiotoxicity in rats. *Life Sci* **48**:675-683.
- Pentel PR and Keyler DE (1988) Effects of high dose alpha-1-acid glycoprotein on desipramine toxicity in rats. *J Pharmacol Exp Ther* **246**:1061-1066.
- Pentel PR and Keyler DE (2004) Vaccines to treat drug addiction, in *New Generation Vaccines* (Levine MM ed) pp 1057-1066, Dekker, New York.

- Pentel PR and Malin DH (2002) Vaccines for nicotine dependence; Targeting the drug instead of the brain. *Respiration* **69**:193-197.
- Pentel PR, Malin DH, Ennifar S, Hieda Y, Keyler DE, Lake JR, Milstein JR, Basham LE, Coy RT, Moon JW, Naso R and Fattom A (2000) A nicotine conjugate vaccine reduces nicotine distribution to brain and attenuates its behavioral and cardiovascular effects in rats. *Pharmacol Biochem Behav* **65**:191-198.
- Proksch JW, Gentry WB and Owens SM (2000) Anti-phencyclidine monoclonal antibodies provide long-term reductions in brain phencyclidine concentrations during chronic phencyclidine administration in rats. *J Pharmacol Exp Ther* **292**:831-837.
- Sanderson SD, Cheruku SR, Padmanilayam MP, Vennerstrom JL, Thiele GM, Palmatier MI and Bevins RA (2003) Immunization to nicotine with a peptide-based vaccine composed of a conformationally biased agonist of C5a as a molecular adjuvant. *Int Immunopharmacol* **3**:137-146.
- Satoskar SD, Keyler DE, LeSage MG, Raphael DE, Ross CA and Pentel PR (2003) Tissue-dependent effects of immunization with a nicotine conjugate vaccine on the distribution of nicotine in rats. *Int Immunopharmacol* **3**:957-970.
- Stewart SS and Fuller SA (1989) Enhancement of hybridoma production by medium supplemented with murine ascitic fluid. *J Immunol Methods* **123**:45-53.
- Tuncok Y, Hieda Y, Keyler DE, Brown S, Ennifar S, Fattom A and Pentel PR (2001) Inhibition of nicotine-induced seizures in rats by combining vaccination against nicotine with chronic nicotine infusion. *Exp Clin Psychopharmacol* **9**:228-234.
- Tyndale RF and Sellers EM (2002) Genetic variation in CYP2A6-mediated nicotine metabolism alters smoking behavior. *Ther Drug Monit* **24**:163-171.

FOOTNOTES

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FIGURE LEGENDS

Figure 1. Serum and brain nicotine concentrations after a single nicotine dose in rats pretreated with 10 mg/kg Control-mAb, monoclonal NICmAbs or Nic-IgG. Nicotine 0.03 mg/kg was administered 30 minutes after the pretreatment and serum and brain collected 3 min after the nicotine dose. Pretreatment with each of the NICmAbs or with Nic-IgG increased nicotine retention in serum and reduced nicotine distribution to brain. Decreasing K_d (values above bars) was associated with increasing nicotine concentration in serum ($r = 0.67$, $p < 0.001$) and decreasing nicotine concentration in brain ($r = 0.69$, $p < 0.001$). ** $p < 0.01$ compared to Control-mAb.

Figure 2. Correlations between the serum unbound nicotine concentration and brain nicotine concentration. Top panel shows data from the four different antibodies, all administered at a dose of 10 mg/kg. Bottom panel shows dose-response data for NICmAb311.

Figure 3. Dose-response relationship for nicotine distribution after treatment with NICmAb311 compared to Control-mAb. Data points for each of 2 rats at each dose are shown. Increasing NICmAb311 dose was associated with increasing serum nicotine concentration measured 3 minutes 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 Figure 1, are shown for comparison.

Figure 4. Serum nicotine concentrations (mean \pm SD) 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.

Table 1. Effects of monoclonal nicotine-specific antibodies and Nic-IgG on nicotine protein binding in serum (mean±SD). The corresponding total serum and brain nicotine concentrations are shown in Figure 1. There was a significant relationship between antibody Kd and both the % nicotine bound ($r = 0.90$, $p < 0.001$) and the unbound nicotine concentration in serum ($r = 0.68$, $p < 0.001$). ANOVA showed that all antibody groups differed from the control group.

Treatments (n = 6)	Kd (nM)	Nicotine % Bound	Unbound Nicotine ng/ml
Control-mAb	---	7±7	21±4
NICmAb079	250	64±7 **	16±3 *
NICmAb810	70	81±5 **	15±4 **
NICmAb311	60	95±1 **	10±1 **
Nic-IgG	1.6	99±0.1 **	3±1 **

* $p < 0.01$, ** $p < 0.01$ ** compared to control group

Table 2. Effects of NICmAb311 dose on nicotine binding in serum. Values are the mean±SD except for unbound nicotine concentrations in the NICmAb groups, which show only the means because there were just 2 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 Figure 3. There was a significant correlation between NICmAb311 dose and both the % nicotine bound ($r = .93, p < 0.01$) and the unbound nicotine concentration in serum ($r = .98, p < 0.05$).

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

Table 3. Noncompartmental pharmacokinetic parameter estimates for nicotine in rats pretreated with NICmAb311 or Control-IgG. Rats received a single i.v. bolus dose of nicotine 0.1 mg/kg. NICmAb311 substantially reduced the Vdss, decreased Cl, and increased the terminal half-life.

Treatment	C_{max} ng/ml	AUC h·ng/ml	V_{dss} L/kg	Cl_t L/h·kg	T_{1/2} hours
Control-IgG	29 ± 12	48 ± 21	3.48 ± 1.24	2.15 ± 0.60	1.1 ± 0.2
NICmAb311	144 ± 33 **	473. ± 120**	0.64 ± 0.10**	0.22 ± 0.05 **	2.5 ± 0.3 ***

p<0.01, *p < 0.001 compared to controls

figure 1

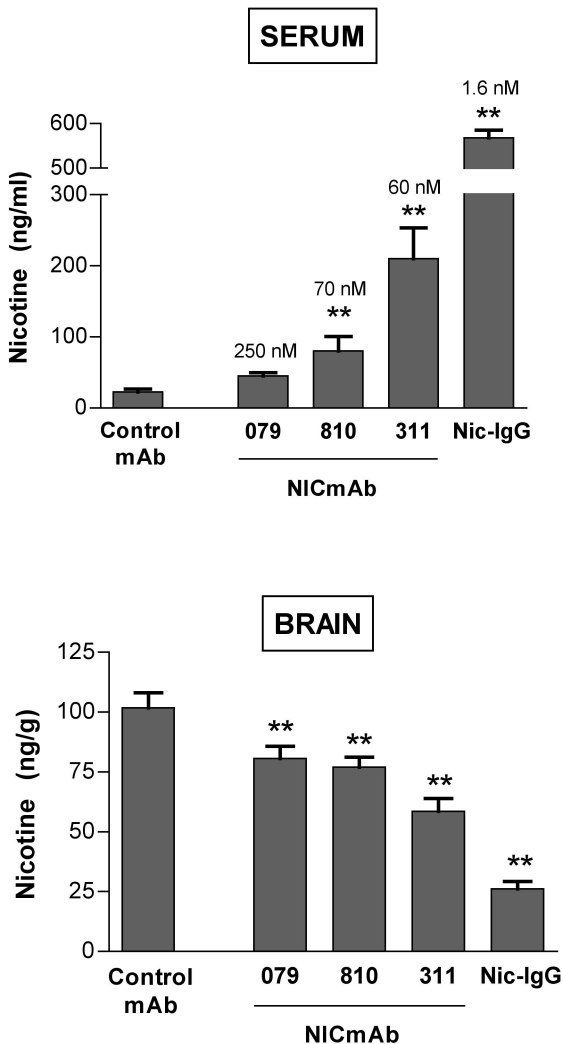


figure 2

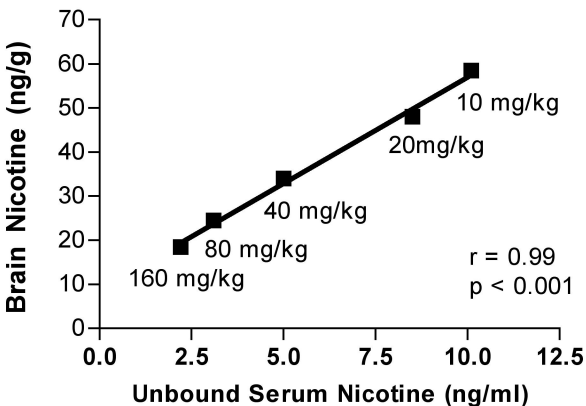
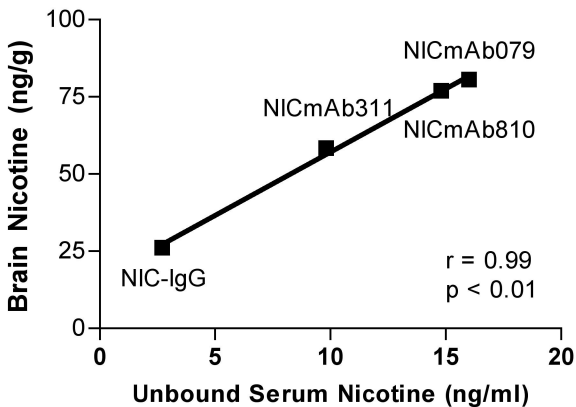


figure 3

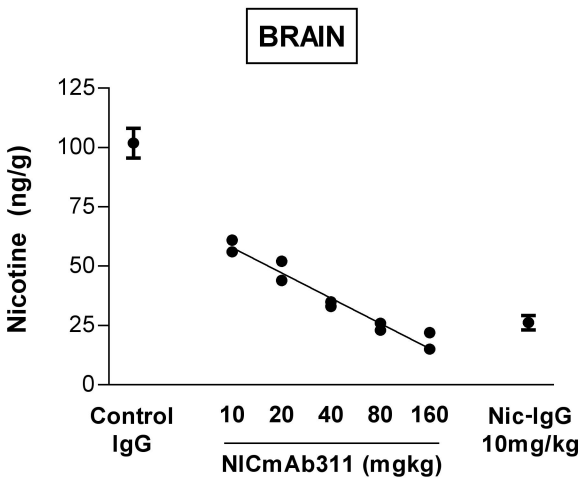
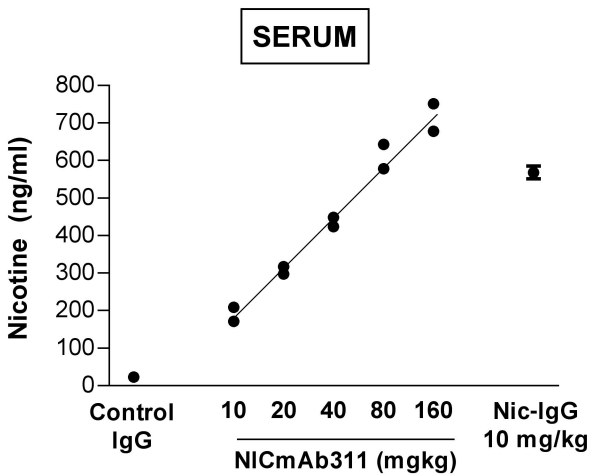


figure 4

