CLINICAL TRIALS

Safety and immunogenicity of a nicotine conjugate vaccine in current smokers

Immunotherapy is a novel potential treatment for nicotine addiction. The aim of this study was to assess the safety and immunogenicity of a nicotine conjugate vaccine, NicVAX, and its effects on smoking behavior. Smokers (N = 68) were recruited for a noncessation treatment study and assigned to 1 of 3 doses of the nicotine vaccine (50, 100, or 200 μg) or placebo. They were injected on days 0, 28, 56, and 182 and monitored for a period of 38 weeks. Results showed that the nicotine vaccine was safe and well tolerated. Vaccine immunogenicity was dose-related ($P < .001$), with the highest dose eliciting antibody concentrations within the anticipated range of efficacy. There was no evidence of compensatory smoking or precipitation of nicotine withdrawal with the nicotine vaccine. The 30-day abstinence rate was significantly different across the 4 doses ($P = .02$), with the highest rate of abstinence occurring with 200 μg. The nicotine vaccine appears to be a promising medication for tobacco dependence. (Clin Pharmacol Ther 2005;78:456-67.)

Dorothy K. Hatsukami, PhD, Stephen Rennard, MD, Douglas Jorenby, PhD, Michael Fiore, MD, MPH, Joseph Koopmeiners, Arjen de Vos, MD, PhD, Gary Horwith, MD, and Paul R. Pentel, MD *Minneapolis, Minn, Omaha, Neb, Madison, Wis, and Rockville, Md*

Surveys show that, although about 41% of smokers make a quit attempt each year, less than 5% of smokers are successful at remaining abstinent for 3 months to a year.[1] Smokers seeking available behavioral and pharmacologic therapies can enhance successful quit rates by 2- to 3-fold over control conditions.[2] However, the rates of abstinence remain relatively low. In clinical trials the long-term rate of abstinence by use of pharmacologic treatments, with or without behavioral therapy, is about 25% on average.[2] Moreover, these percentages most likely exaggerate the efficacy of intervention because these trials are typically composed of subjects who are highly motivated to quit and who are free of complicating diagnoses such as depression or other psychiatric disorders.[3] Because the vast majority of those who attempt to quit will not achieve prolonged abstinence, the need for better approaches to smoking cessation is evident.

The potential usefulness of vaccination as an intervention for drug abuse was first demonstrated in an animal model by Bonese et al[4] for heroin and was subsequently extended in animal models to phencyclidine, cocaine, methamphetamine (INN, metamfetamine), and nicotine (see reference 5). Vaccination elicits the production of drug-specific antibodies that bind drug and reduce its distribution to the brain. Preclinical studies of short- and long-term administration of clinically relevant doses of nicotine show that nicotine vaccines reduce the distribution of nicotine to the brain in rats by up to 65%.[6] Vaccination of rats or passive immunization with nicotine-specific immunoglobulin G (IgG) has also been shown to reduce nicotine discrimination, the devel-

From the University of Minnesota Medical School, Cancer Center, Tobacco Use Research Center, and Minneapolis Medical Research Foundation, Minneapolis; University of Nebraska Medical School, Omaha; University of Wisconsin Medical School, Center for Tobacco Research and Intervention, Madison; and Nabi Biopharmaceuticals, Rockville.

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Reprint requests: Dorothy K. Hatsukami, Tobacco Use Research Center, 2701 University Ave SE, Suite 201, Minneapolis, MN 55414.

E-mail: *hatsu001@umn.edu*

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opment of nicotine dependence, the reversal of nicotine abstinence signs by nicotine, the reinstatement of nicotine self-administration, and the acquisition and maintenance of nicotine self-administration.[7-11] These data provide support for the hypothesis that immunization can impact nicotine drug effects, including those responsible for maintaining nicotine addiction.

The general concept of vaccination as a means of blocking nicotine's effects is attractive because of its specificity. The antibody itself does not appreciably enter the brain so that central nervous system side effects are not expected. The antibodies elicited by vaccination are highly specific for nicotine and do not bind other ligands, endogenous neurotransmitters, or receptors. Thus a vaccine against nicotine should not be associated with many of the adverse effects inherent to other pharmacologic treatments for drug abuse. Vaccination for nicotine dependence would then represent a unique addition to the available strategies for treating nicotine dependence.

The primary aims of this study were to assess the safety and immunogenicity of an investigational conjugate nicotine vaccine. Several concerns, such as the potential for compensatory smoking to overcome the reduction in the reinforcing effects from smoking as a result of the binding of nicotine to antibody, as well as a precipitation of withdrawal symptoms after vaccination, have been raised regarding immunotherapy for smoking cessation.[12] Therefore secondary objectives of the study were to assess withdrawal symptoms, the occurrence of compensatory smoking behavior, and change in tobacco use. Multiple administrations of 50, 100, or 200 μg of a nicotine conjugate vaccine were delivered to current smokers in a study in which neither desire to quit nor willingness to make a quit attempt was required.

METHODS

Subjects

A total enrollment of 63 subjects was planned, and 68 subjects (15 women and 53 men) were enrolled from 3 research sites: University of Minnesota, Minneapolis, Minn (n = 24); University of Wisconsin, Madison, Wis (n = 21); and University of Nebraska, Omaha, Neb (n = 23). The 5 additional subjects replaced subjects who withdrew from the study within 1 month after the first injection. All withdrawn subjects did so for non–safety-related reasons. Each site recruited subjects through advertisements in newspapers or on the radio or television. Interested participants were asked to call the research clinic, the study was explained, and an initial screening was undertaken. If subjects met the initial screening criteria, they were scheduled for an appointment to obtain informed consent and to undergo a comprehensive screening of their physical health. Major inclusion criteria for the study included the following: (1) male subjects or female subjects who were not of child-bearing potential and were aged at least 18 years, (2) subjects currently smoking at least 15 cigarettes per day (with alveolar carbon monoxide level of >10 ppm), (3) a history of smoking for at least the past 6 months without a period of abstinence for greater than 7 days and no intention to quit for the next 30 days, (4) good general health (ie, without significant medical history, clinically significant physical examination findings, clinically significant electrocardiographic findings, or 1-second forced expiratory volume <75% and forced expiratory vital capacity <80% of the predicted mean value for height, age, race, and gender), and (5) good mental health (ie, no history of alcohol or drug abuse or psychiatric history within the past 3 months). Major exclusion criteria were as follows: (1) any prior exposure to a nicotine vaccine or exposure to any other vaccine, except for influenza, 30 days before administration of study product; (2) allergy to alum or any components of the vaccine; (3) use of inhaled corticosteroids, systemic steroids, antihistamines, or immunosuppressive agents within 30 days before the study; and (4) use of any smoking cessation technique within 30 days of the screening visit. The study was conducted under an Investigational New Drug application to the US Food and Drug Administration. The institutional review boards at the study sites approved the study protocol and informed consent forms.

Table I. Time and events schedule

Study day	*Events*
−10	Baseline visit
0	Baseline visit, injection 1
1	Safety evaluation
7, 14, 21	Clinic visits
28	Injection 2
29	Safety evaluation
35, 42, 49	Clinic visits
56	Injection 3
57	Safety evaluation
63, 70, 77, 98, 126, 154	Clinic visits
182	Injection 4
183	Safety evaluation
189, 203, 231, 266	Clinic visits

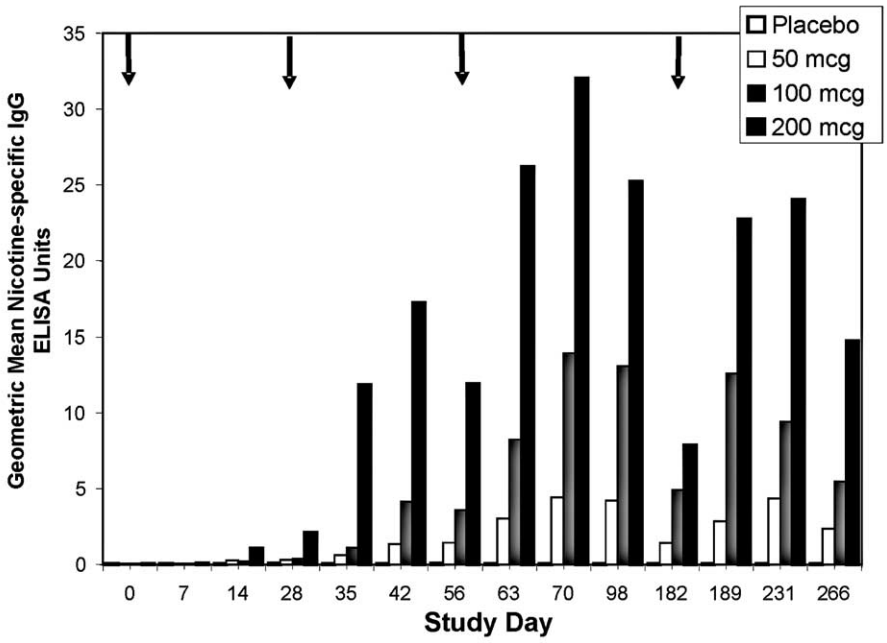

Fig 1. Mean antibody concentration by nicotine vaccine dose over time. *Arrows* indicate vaccine injection times. IgG, Immunoglobulin G; ELISA, enzyme-linked immunosorbent assay.

Study design

This was a multicenter, randomized, double-blinded, placebo-controlled, parallel-arm, dose-comparison study. Participants were randomized to 1 of 3 treatment groups, as follows: 50 μg vaccine or matching placebo, 100 μg vaccine or matching placebo, or 200 μg vaccine or matching placebo. Within each treatment group, 21 subjects were randomized in a ratio of 2:1 (vaccine/placebo), yielding a total of 14 vaccine and 7 placebo subjects per treatment group. For each subject, there was a 10-day screening period followed by randomization and active participation for 38 weeks (9 months) from day 0 (Table I). Generally, the clinic visits occurred weekly for the first 12 weeks and then approximately monthly for the next 26 weeks.

Injections were scheduled to be administered at days 0, 28, 56, and 182. A physician or study nurse administered all investigational products. After the injections, each subject remained at the study site for a minimum of 30 minutes for recording of postinjection vital signs and for observation. Subjects returned to the clinic for follow-up evaluation on the next day after each injection. The data and safety monitoring board reviewed all available postinjection subject data before each subsequent administration.

No instructions were given to the subjects regarding their smoking behavior. If they inquired about their smoking, they were informed to smoke as they felt comfortable. No behavioral counseling for cessation was provided unless the participant indicated that they would like to quit smoking. If a study participant expressed a desire to quit, a standardized treatment protocol was implemented, in which subjects received brief treatment intervention and a treatment manual. Otherwise, the sessions were focused primarily on assessment of safety and tobacco use behaviors.

Study outcome measures

Safety. Safety was assessed through physical examinations, vital signs, 12-lead electrocardiography, and laboratory studies including hematologic testing, chemical testing, and urinalysis, as well as through monitoring of "reactogenicity" and adverse events. Reactogenicity events included local reactions (eg, erythema, induration, tenderness, ache, burning, and heat) and systemic complaints (malaise, myalgia, nausea, vomiting, fever, and headache) that occurred within the first 7 days after injection. By use of an electronic diary, the presence and severity of these 12 potential events were assessed and recorded daily by subjects (rated as not present, mild, moderate, or severe). Adverse events were monitored at each clinic visit by querying the subjects about any health problems that they may have had since the last visit. Withdrawal symptoms were assessed weekly, or daily for

2 weeks after a quit attempt and then weekly, by use of the Minnesota Nicotine Withdrawal Scale.[13]

Immunogenicity. Blood specimens were collected during the study (Fig 1) for determination of antinicotine antibody levels measured by enzyme-linked immunosorbent assay (ELISA). Microtiter plates were coated with 3′-aminomethylnicotine conjugated to polyglutamic acid and blocked with 1% nonfat dry milk in phosphate-buffered saline solution. Human sera, reference standards, and controls were applied to the plate in 2-fold dilutions and incubated for 45 minutes at 37°C. After a wash step, the plates were incubated with horseradish peroxidase–labeled goat antihuman IgG for 30 minutes at 37°C. After a final wash step, the color was developed by addition of peroxidase substrate. Plates were read at A_{450}. The amount of nicotine-specific IgG was quantitated in samples by comparison with the standard curve. The nicotine human IgG ELISA detects all IgG subtypes.

Because no national or international reference standards exist for nicotine antibodies, reference standards were developed by Nabi Biopharmaceuticals (Rockville, Md) and prepared from pools of serum from human volunteers who had been vaccinated with NicVAX. Nicotine-specific IgG antibody was quantitated by an ELISA in which antibody bound to nicotine-coated plates was quantitated against antibody bound by anti–Fab-coated plates.

Assessment of smoking behavior and effects

A tobacco use history form was completed during the screening to obtain information on characteristics of the subject's tobacco use behavior and to assess dependence by use of the Fagerstrom Test for Nicotine Dependence.[14] A daily electronic diary, consisting of a set of questions inquiring about cigarette and other tobacco use, was completed by subjects to assess changes in smoking behaviors, including compensatory smoking. Biochemical measures of smoke intake were obtained by assessing urinary cotinine concentration and concentrations of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides (NNAL-Gluc). NNAL and NNAL-Gluc (total NNAL) are metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and are highly correlated with cotinine. Total NNAL was measured because cotinine concentrations in serum may be altered by vaccination and may not be an accurate measure of nicotine intake in vaccinated subjects.[15] The first morning urine voids were collected, processed, and quantified for NNAL and NNAL-Gluc as described previously.[16,17] Measurements for total NNAL were obtained at each injection time and at the end of the study. Spot urine samples were collected and quantified for cotinine and its glucuronides (total cotinine) as described previously.[16] Measurements for total cotinine were obtained before injection, at each injection time, weekly for 3 weeks after the injection, and at the end of the study. The quantification was conducted at the University of Minnesota. Alveolar CO was measured by use of the Micro Smokerlyzer (Bedfont, Medford, NJ) and was obtained at each clinic visit.

Product descriptions

The investigational product, AMNic-rEPA (NicVAX), consists of the hapten 3′-aminomethylnicotine conjugated to recombinant *Pseudomonas aeruginosa* exoprotein A (rEPA). Each single-use vial contained 3′-aminomethylnicotine conjugated to 100 μg rEPA adsorbed to 0.8 mg Alhydrogel 85, an adjuvant, in 1 mL phosphate-buffered saline solution (0.15-mol/L sodium chloride; 0.002-mol/L sodium phosphate, pH 7.2; 0.01% polysorbate 80). Placebo contained phosphate-buffered saline solution with 0.8 mg Alhydrogel 85 (E.M. Sergeant Pulp and Chemical Co, Inc, Clifton, NJ). To maintain blinding, each subject received 2 injections of 1 mL on each injection day (1 injection in each deltoid muscle).

Statistical analyses

Data were analyzed primarily by the Biostatistics Core of the University of Minnesota Transdisciplinary Tobacco Use Research Center and by Nabi Biopharmaceuticals. Subject data were analyzed up to the point of discharge from the study or up to the point of dropping out of the study. Primary and secondary outcomes were compared across doses and antibody concentration levels. Antibody concentration levels were determined by the quartile of each subject's maximum antibody concentration. Baseline characteristics were compared by doses and antibody concentration levels by use of 1-way ANOVA or Fisher exact test as appropriate. Subsequent analyses were adjusted for baseline differences significant at the $P \leq .05$ level unless otherwise indicated.

Antibody concentration was compared by dose by use of the maximum antibody concentration achieved for each subject. The geometric mean maximum antibody concentration was compared by dose levels by use of a general linear model to adjust for baseline differences. Geometric mean antibody concentrations were compared for the total study population over time by use of a general linear mixed model to account for the correlation among repeated measurements within subjects.[18]

Table II. Demographics and smoking history

Variable	*Total sample*	*Placebo*
Age (y)	43.8 (41.5-46.0)	46.8 (43.2-50.4)
Male (No.)	53	19
Female (No.)	15	4
Ethnicity (No.)		
White	61	20
Other	7	3
No. of cigarettes smoked		
1-15	6	4
16-25	39	10
≥26	18	7
Subjects' mean age at first cigarette (y)	15.6 (14.7-16.5)	16.0 (14.2-17.8)
No. of serious quit attempts	6.2 (4.7-7.7)	5.7 (3.6-7.8)
Mean FTND score	5.9 (5.3-6.4)	6.0 (5.2-6.9)
Baseline cotinine concentration (ng/mL)	5070 (4300-5840)	4630 (3200-6070)

Data are given as mean and 95% confidence interval or as actual value.
FTND, Fagerstrom Test for Nicotine Dependence.

Nicotine withdrawal (sum score of the withdrawal symptoms minus the craving item) and craving and compensatory smoking (as determined by change in number of cigarettes per day, CO levels, and total cotinine concentration) data were also compared at 28-day intervals after the second, third, and fourth injections. Because of the limited collection times for the assessment of total NNAL concentrations, the samples from the interval from the second injection to the end of the study were compared. At each interval, the mean and maximum values were determined for each subject and the difference from baseline was calculated (mean minus baseline, maximum minus baseline). For most measures (ie, withdrawal symptoms, craving, cigarette intake), baseline was calculated as the 4 weeks after the first injection, except for CO and cotinine, for which we had preinjection baseline data. The 4 weeks after the first injection was chosen to reflect baseline levels because of the minimal increase in antibody concentrations observed during this time. These differences were compared by doses and antibody concentration levels by use of a general linear model to adjust for significant baseline covariates.

Abstinence rates were compared by vaccine doses and antibody concentration levels by use of the Fisher exact test. Time to first 30-day quit attempt was summarized by Kaplan-Meier curves and compared by use of the log-rank test. Censored observations represent subjects who did not achieve 30 days of consecutive abstinence before the end of the study or who were lost to follow-up. These analyses were not adjusted for baseline differences because of the small number of events.

RESULTS

Subjects

A total of 130 subjects were screened for the study. Of these participants, 62 did not meet screening criteria and 68 were admitted to the study. Of the 68 subjects who constituted the analysis sample, 61 received the second injection, 60 received the third, and 56 received the fourth. The reasons for study withdrawal were as follows: 6 withdrew consent (3 for work-related reasons and 3 for personal reasons), 1 was noncompliant with the protocol, 3 were lost to follow-up, and 2 had medical problems develop unrelated to the study drug. For those subjects who terminated early, the mean number of days (±SD) that they were in the study was 82.2 ± 71.7 (range, 6.0-198.0 days).

A summary of the demographics and smoking histories of the study participants is given in Table II. There were significant differences among dose groups for gender ($P = .04$) and number of cigarettes smoked ($P = .05$). These variables were controlled for in subsequent analyses. There was a significant difference in age among subjects when stratified by quartile of antibody response ($P = .004$), and therefore analyses by antibody concentrations were adjusted by age.

Safety

Table III shows the number of subjects in each dose group with local reactogenicity events. The majority (99.5%) of these events were mild or moderate in severity, with ache and tenderness being the most commonly reported events. There were no significant differences in frequency of local reactogenicity events between placebo

50 μg	*100 μg*	*200 μg*
44.3 (39.7-48.9)	43.3 (38.7-47.9)	39.4 (34.5-44.3)
7	12	15
7	3	1
13	14	14
1	1	2
0	1	1
10	6	13
3	7	1
16.2 (14.0-18.4)	15.3 (14.0-16.6)	14.6 (12.9-16.3)
7.4 (3.4-11.3)	6.1 (2.3-9.8)	6.0 (3.2-8.8)
6.3 (5.2-7.4)	6.1 (5.2-7.1)	4.9 (3.7-6.2)
5270 (3970-6580)	5950 (3840-8960)	4700 (3470-5930)

and each of the dose groups. Table IV shows the results for systemic reactogenicity events across the various dose groups. The majority (89%) of these events was mild, with general discomfort or malaise, headache, and myalgia being the most commonly reported events. There were no significant differences in frequency of systemic reactogenicity events between placebo and each of the dose groups. All local and systemic reactogenicity events resolved within days without medical intervention. The mean number of days for local reactogenicity events across doses ranged from 1.3 to 1.8 days (95% confidence interval, 1.2-2.1 days), and the mean number of days for systemic reactogenicity events ranged from 1.5 to 2.8 days (95% confidence interval, 1.3-3.8 days).

The adverse events occurring in more than 10% of the subjects within each of the treatment groups are detailed in Table V. A total of 345 adverse events were reported by 64 of 68 subjects. The vast majority (92.5%) of these events were mild or moderate in severity. In 7% of nicotine vaccine recipients, these adverse events were considered to be related to study drug, versus 5% in placebo recipients. The most frequently reported events were upper respiratory tract infection, headache, cough, and nasopharyngitis. No differences were observed in the frequency of these adverse events among the 4 treatment groups. Two serious adverse events were reported, neither of which was related to study drug. One subject (in the 50-μg group) underwent a hysterectomy because of postmenopausal menorrhagia and withdrew from the study. The other subject (in the 100-μg group) was admitted to the hospital for evaluation of complaints of chest pain and for subsequent coronary artery stent placement. This subject had a history of chest pain (which he reported had been diagnosed as noncardiac) that predated his study participation.

Experiencing precipitate withdrawal symptoms and craving as a result of nicotine injection was also con-

Table III. Number of subjects* with local reactogenic events

	Nicotine vaccine dose			
Event	*Placebo (n = 23)*	*50 μg (n = 14)*	*100 μg (n = 15)*	*200 μg (n = 16)*
Ache	21	11	14	15
Burning	8	5	7	7
Heat	5	4	6	3
Redness	7	4	6	4
Induration	8	3	8	4
Tenderness	21	11	14	14

*Each subject was counted once because a subject may have had multiple reports of the same reactogenicity event.

Table IV. Number of subjects* with systemic reactogenicity events

	Nicotine vaccine dose			
Event	*Placebo (n = 23)*	*50 μg (n = 14)*	*100 μg (n = 15)*	*200 μg (n = 16)*
Fever	6	2	3	3
Malaise	16	8	10	12
Headache	17	8	11	14
Myalgia	17	8	10	13
Nausea	7	4	4	3
Vomiting	1	0	0	0

*Each subject was counted once because a subject may have had multiple reports of the same reactogenicity event.

Table V. Incidence of adverse events in 10% of subjects or greater

	Nicotine vaccine dose			
Body system/event	*50 μg (n = 14)*	*100 μg (n = 15)*	*200 μg (n = 16)*	*Placebo (n = 23)*
Gastrointestinal disorders				
Diarrhea	0 (0.0)*	1 (6.7)	3 (18.8)	2 (8.7)
Nausea	2 (14.3)	1 (6.7)	1 (6.3)	2 (8.7)
Toothache	0 (0.0)	3 (20.0)	1 (6.3)	0 (0.0)
Vomiting	0 (0.0)	0 (0.0)	2 (12.5)	2 (8.7)
General disorders and administration site conditions				
Fatigue	0 (0.0)	2 (13.3)	1 (6.3)	0 (0.0)
Infections and infestations				
Viral gastroenteritis	0 (0.0)	0 (0.0)	0 (0.0)	4 (17.4)
Sinusitis	2 (14.3)	1 (6.7)	3 (18.8)	1 (4.3)
Upper respiratory tract infection	6 (42.9)	4 (26.7)	1 (6.3)	8 (34.8)
Injury				
Joint sprain	0 (0.0)	0 (0.0)	2 (12.5)	2 (8.7)
Musculoskeletal and connective tissue disorders				
Muscle cramp	0 (0.0)	3 (20.0)	0 (0.0)	0 (0.0)
Musculoskeletal stiffness	1 (7.1)	0 (0.0)	2 (12.5)	0 (0.0)
Neck pain	0 (0.0)	2 (13.3)	1 (6.3)	1 (4.3)
Pain in limb	0 (0.0)	0 (0.0)	2 (12.5)	0 (0.0)
Nervous system disorders				
Dysgeusia	1 (7.1)	3 (20.0)	3 (18.8)	4 (17.4)
Headache	2 (14.3)	6 (40.0)	4 (25.0)	6 (26.1)
Psychiatric disorders				
Insomnia	0 (0.0)	0 (0.0)	3 (18.8)	2 (8.7)
Respiratory disorders				
Cough	1 (7.1)	2 (13.3)	4 (25.0)	3 (13.0)
Nasal congestion	1 (7.1)	1 (6.7)	3 (18.8)	1 (4.3)
Nasopharyngitis	1 (7.1)	7 (46.7)	5 (31.3)	6 (26.1)
Sinus congestion	0 (0.0)	2 (13.3)	4 (25.0)	1 (4.3)

*Values are number and percent. Percent is based on total number of subjects within treatment group.

sidered a potential side effect from the nicotine vaccine. Analysis showed no significant differences in mean maximum or mean change in withdrawal or craving after the second, third, and fourth injections compared with the preinjection baseline values when subjects were stratified by dose or by quartiles of antibody concentrations ($P = .19$-$.89$). When subjects were divided into quitters (abstinent for 30 days with CO $\leq$8 ppm) and nonquitters (not abstinent for 30 days at any time during the study), the changes from baseline in mean withdrawal scores and craving for nonquitters were not significantly different across the 4 dose levels ($P = .30$ and $P = .89$, respectively) (see Fig 2, top row). For the quitters, there was a significant decrease in mean craving observed during the first 2 weeks of abstinence compared with baseline ($P = .004$), but the decrease in withdrawal symptoms was not significant ($P = .10$). Comparisons across dose levels for quitters were not completed because of the small number of quitters.

Immunogenicity

Fig 1 shows the geometric mean antinicotine antibody concentrations over time. There was no significant antibody detected after the first (priming) injection. For the 3 subsequent doses, there were significant dose ($P < .001$) and time ($P < .001$) effects, as well as a significant dose-by-time interaction ($P < .001$). For all nicotine vaccine groups, there were sharp increases in geometric mean antibody levels after the second and third injections, with peak concentrations after the third injection. Fig 3 shows the geometric mean maximum

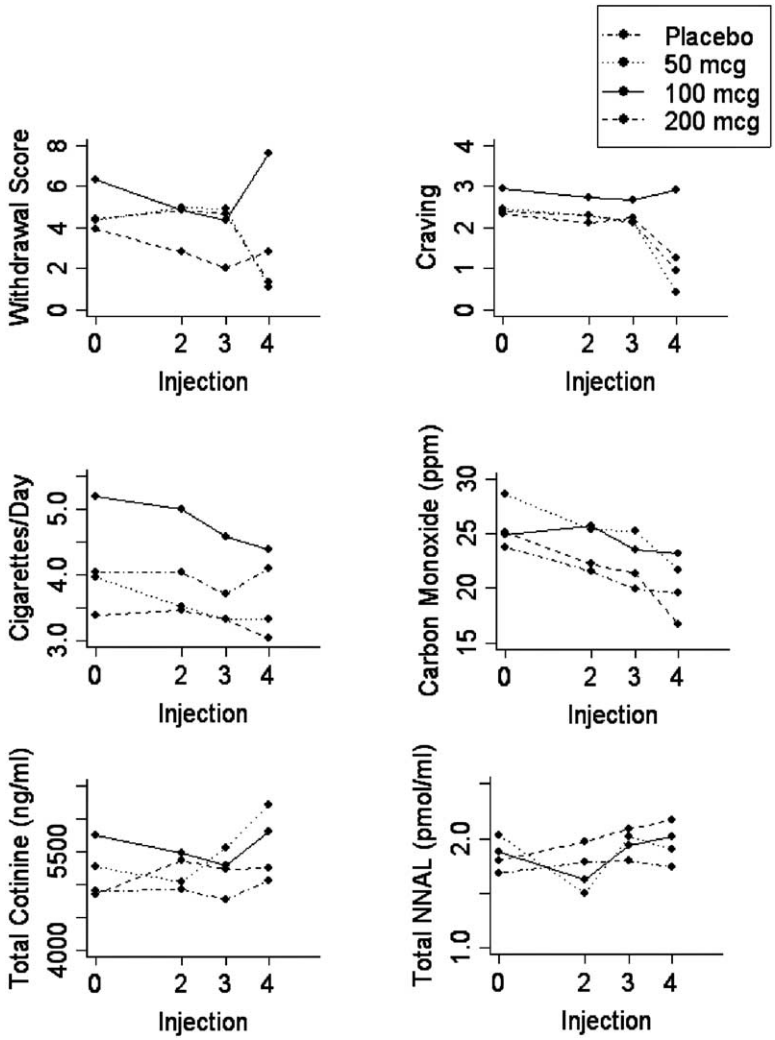

Fig 2. Mean withdrawal symptom scores, craving, number of cigarettes per day, carbon monoxide level, and concentrations of total cotinine and total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) after each injection period among nonquitters (not having been abstinent for a 30-day period of time) by vaccine dose.

antibody levels by dose of vaccine. Significant differences in antibody concentrations were observed ($P <$ .001).

Smoking intake (focus on compensatory smoking behavior)

No significant differences were observed in mean or mean maximum change in cotinine concentration or total NNAL concentrations after the second, third, and fourth injections compared with baseline across doses and across quartiles of antibody concentration levels (P = .14-.94). A significant difference was observed in mean (P = .04) and mean maximum (P = .04) change in number of cigarettes by antibody concentration levels, with the greatest reductions being observed in the second-quartile antibody concentration after the third injection. In addition, significant effects were observed for mean change in CO by concentration levels after the second injection (P = .04), with the greatest reductions being observed in the second-quartile antibody concentration. No differences for CO or number of cigarettes were observed across doses (P = .20-.99).

Data were examined for change compared with baseline in mean number of cigarettes per day, CO, and total cotinine and NNAL for nonquitters, as well as change in mean total NNAL and total cotinine for

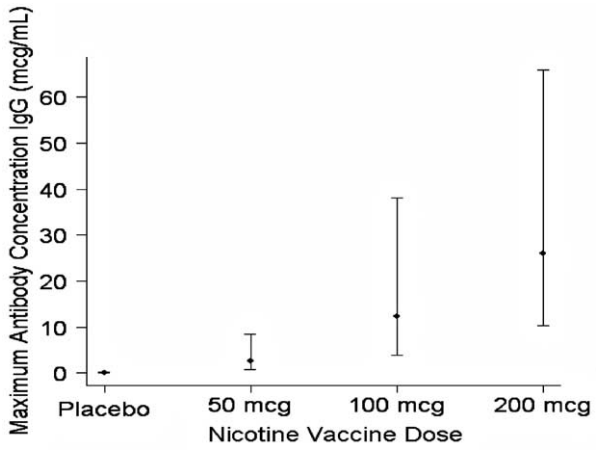

Fig 3. Mean maximum antibody concentration by nicotine vaccine dose.

quitters. For nonquitters, the mean changes from baseline for these variables were not significantly different across the 4 dose levels (P = .32-.96) (Fig 2). Among quitters, significant reductions were observed for total cotinine (P = .007) but not for total NNAL (P = .11), possibly as a result of NNAL's long half-life relative to the duration of abstinence at the time of assessment.

Effects on quitting behavior

There was a significant dose effect for the number of subjects who achieved 30-day biochemically confirmed (CO ≤8 ppm) abstinence (6, 1, 0, and 2 subjects in the 200, 100, and 50 μg vaccine and placebo groups, respectively; P = .02). Analysis of abstinence by quartile of antibody concentrations showed a trend in the same direction (P = .08). There was also a significant dose effect in time to achieve 30-day abstinence (P = .01) (Fig 4), with the shortest interval occurring for the group receiving 200 μg vaccine.

DISCUSSION

The results from this study show that multiple vaccine doses of 50 to 200 μg AMNic-rEPA per dose are safe and well tolerated in healthy smokers. The comparable frequency of reactogenicity events in the placebo and vaccine groups suggests that these events were a result of the administration of either buffer or adjuvant and not the active vaccine components. Published[19] or preliminary[20] data for 2 other nicotine vaccines and published data for a cocaine vaccine[21] have shown similar safety profiles. There was no evidence of precipitation of withdrawal symptoms in the total sample, in nonquitters, or in quitters. It is likely that the development of antibody concentrations occurs at a sufficiently slow rate that withdrawal symptoms are not evoked. Furthermore, vaccination of rats against nicotine prolongs the half-life of nicotine by 3- to 6-fold,[22] resulting in a more gradual decrease in nicotine concentrations. If this alteration in half-life also occurs in humans, the slower decline in nicotine levels could minimize the occurrence of withdrawal symptoms.

A clear dose-response relationship was observed between the dose of nicotine vaccine and the mean antibody concentrations in serum, although the range of serum antibody concentrations was wide. In the study conducted with the cocaine vaccine, antibody levels varied by 31.9-fold at the 56-day assessment point,[21] which is similar to the considerable range observed for the nicotine vaccine. As expected, the nicotine-specific antibody concentration in serum was low after the first vaccine dose but increased with the second and third doses and responded to a booster dose at 6 months. Antibody levels declined between injections, even though most subjects continued to smoke, because nicotine itself is not a complete immunogen. The rate of antibody decline could not be accurately determined in this study because too few antibody levels were measured between any 2 doses, but the data suggest that repeated booster doses may be needed to maintain maximal antibody levels for more than a few months and that any effects of immunization would not be permanent. These results are generally similar to those reported in a phase I study of a nicotine vaccine using a similar hapten (nicotine with a linker at the 3′ position) conjugated to a viruslike particle.[19] In nonsmokers, antibody titers increased after each of 2 vaccine doses and antibody titers declined after the second injection, with an estimated half-life of 57 days. As in our study, the range of antibody titers achieved was wide, indicating considerable interindividual variability in the antibody titer response.

The efficacy of vaccination in rats, as measured by its ability to reduce nicotine distribution to the brain, is closely related to the serum antibody concentration.[22] The best responders in our study, primarily in the group receiving 200 μg vaccine, had antibody levels comparable to those that have been associated with substantial changes in nicotine distribution in rats (>30 μg/mL).[23] Thus our study is encouraging with regard to vaccine immunogenicity, but strategies to increase and to sustain this response may be helpful in maximizing the ability of this vaccine to attenuate nicotine's effects in humans.

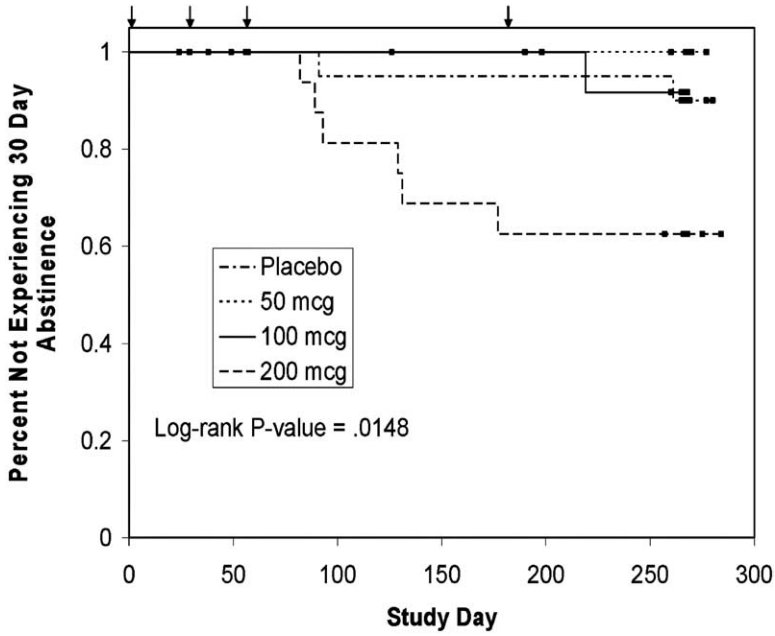

Fig 4. Survival curve for subjects not experiencing a 30-day abstinence period. *Solid squares* indicate censored data points of subjects who did not achieve 30 days of abstinence before the end of treatment or who were lost to follow-up. *Arrows* indicate vaccine injection times.

There was a concern that vaccination of smokers might result in compensatory smoking as a result of the reduction in the amount of nicotine reaching the brain. However, our study showed no increase in smoking as measured by self-reported number of cigarettes per day, CO level, and NNAL concentrations in the total sample or among nonquitters. Cotinine concentrations also did not show compensation but should be interpreted cautiously because this vaccine decreases nicotine clearance in rats and, presumably, the rate of cotinine formation as well. Changes in cotinine levels may reflect altered nicotine metabolism rather than intake.[22] Significant differences across antibody concentration quartiles for number of cigarettes per day and CO level may be spurious because no clear dose-response pattern was observed. The results did not, however, show evidence of increased exposure but rather showed a decrease in exposure that was greatest in those participants with the second-quartile antibody concentration. Smoking behavior was not reported in the only other published study of a nicotine vaccine.[19]

Although cessation efficacy was not a primary endpoint for this study, a significantly greater 30-day abstinence rate was observed in the group receiving the 200-μg vaccine dose compared with all other groups. The fact that this higher abstinence rate was seen only in the highest-dose vaccine group suggests a relationship between efficacy and antibody concentration and is consistent with animal data showing a close correlation between nicotine-specific antibody concentrations elicited by vaccination and magnitude of effects. It is possible that vaccination doses or schedules eliciting even higher antibody concentrations would be still more effective. However, caution is needed in interpreting this result because efficacy was not the primary outcome for this study, the number of subjects was small, and the results may not be predictive of efficacy observed in a large clinical trial.

In summary, the results from this preliminary investigation showed that the conjugate nicotine vaccine studied is safe and well tolerated. Vaccine immunogenicity was dose-related, with the highest dose eliciting serum antibody concentrations within the anticipated target range for efficacy. The large individual variability in immunogenicity could present a challenge to the clinical use of the vaccine, and further efforts to maximize immunogenicity may facilitate further clinical testing of this strategy for treating tobacco addiction. Future clinical trials should also consider the effects of gender on treatment efficacy because of evidence showing potential differences in sensitivity to nicotine's effects.[24-26]

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In the past few years, Dr Rennard has had a number of relationships with companies who provide products or services relevant to outpatient management of chronic obstructive pulmonary disease. These relationships include serving as a consultant, advising regarding clinical trials, speaking at continuing medical education programs, and performing funded research at both basic and clinical levels. Specific companies include the following: Abbott, Amersham, AstraZeneca, Aventis, Bayer, Boehringer Ingelheim, Byk Gulden-Altana, Centocor, Elan, Genaera, GlaxoSmithKline, GloboMax, Inspire Pharmaceuticals, Novartis, Ono Pharma, Otsuka, Pfizer, Philip Morris, RJ Reynolds, Roche, Sanofi-Synthelabo, and Schering-Plough. He does not own stock in any pharmaceutical companies. In the past 5 years, Dr Jorenby has received research support from GlaxoSmithKline, Pfizer, and Sanofi. In the past 5 years, Dr Fiore has served as a consultant, given lectures, or has conducted research sponsored by GlaxoSmithKline, Pharmacia, Pfizer, and Sanofi-Synthelabo. In 1998 the University of Wisconsin appointed Dr Fiore to a named Chair, made possible by an unrestricted gift to the university from GlaxoWellcome. Drs de Vos and Horwith work for Nabi Biopharmaceuticals and own company stock. Dr Pentel received grant support from Nabi Biopharmaceuticals more than 5 years ago. The other authors have no conflicts of interest to declare.

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