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Study of Human Therapeutic Morphine Vaccine: Safety and Immunogenicity

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Abstract: For the economic purpose of clinical trial and study of safety and immunogenicity of therapeutic morphine vaccine, 102 out of 200 outpatient volunteer addicts, whom were interested in abstinence, were injected with morphine vaccine, by randomized double blind method and under placebo control. The volunteers were divided into 3 cohorts, each consists of 30 subjects. The cohorts 1, 2 and 3 were injected with 12.5, 100 and 600 μg mL^{-1} of morphine vaccine, respectively. In each cohort, four additional subjects were injected with placebo. All the volunteers were bled prior to each injection and they got intra deltoid injections at 0-30-60 days and were monitored for safety and antibody production, for 12 months. All of 102 volunteers completed the course of three injections and all of them returned for the final scheduled visit at day 90th. The rise of antibody against morphine in all three vaccinated cohorts was controlled along the 5, 7, 9, 11 and 12 months. The vaccine was well tolerated with dose related increases in antibody levels and had no serious drug-related adverse events. Only 5 persons at the highest dose experienced brief post injection twitching. Anti-morphine antibody was detected by ELISA method after the first injection of 100, 600 μg mL^{-1} and second injection of 12.5 μg mL^{-1} doses and reached to its peak in 3 months and did not decline to baseline after one year. Thus, vaccine was well tolerated with dose related increases in antibody levels and a high proportion of outpatient volunteer addicts were recovered.

Key words: Therapeutic morphine vaccine, safety, immunogenicity

Introduction

Investigation on finding a pharmacotherapeutic agent to treat morphine dependence was begun in the early 1983 in different laboratories such as: The Reference Laboratory of Tehran-Iran, department of biochemistry and control of biological materials. Thereafter, in 1986 in the department of biochemistry of Medical Sciences University of Iran in Tehran morphine vaccine was generated with very expensive method. In 1991 onward, investigation on the morphine vaccine was continued in order to economize the method and we did it at the department of Pilot Biotechnology of Pasteur Institute of Iran (Akbarzadeh *et al*., 1999). In 1995, we finished all clinical trials of Therapeutic Morphine Vaccine on laboratory animals conforming vaccine control protocols of WHO and Iranian National Food and Drug Control Department. In 2000, we began to study the clinical trial of therapeutic morphine vaccine on 1240 addicted persons in Iran. All clinicians and researchers realized that standard drug counseling and struggle of all people, governments and family have just little impact on the addiction of many morphine abusers throughout Iran and the world. The National Secretary on drug abuse estimates that at least, there are 3 million morphine abusers in Iran. The addicted people are

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dispersed throughout the world. While morphine abusers will have no sever life-threatening symptoms when stopping morphine after being vaccinated with therapeutic morphine vaccine, the psychological addiction in people using morphine and disorders can be disabling and led to relapse, but therapeutic morphine vaccine can solve this problem (Akbarzadeh *et al*., 2002; Kosten *et al*., 2000). A therapeutic morphine vaccine provides a unique approach to the pharmacotherapy of morphine addiction. The idea behind a therapeutic morphine vaccine is that, if an addict takes morphine after being immunized, the morphine will encounter and binds to catalytic anti-morphine antibodies on entering the bloodstream, preventing uptake of morphine across the blood-brain barrier systems and dulling or even obliterating the euphoric rush. However, a therapeutic morphine vaccine based on active-immunization has the potential to provide long lasting clinical efficacy for relapse prevention after administration and to have less problems with compliance in humans who are motivated to stop using morphine. Such a therapeutic morphine vaccine has been developed by Pasteur Institute of Iran: therapeutic morphine vaccine comprises a protein conjugate in which morpine-6-succinyl is coupled to the carrier protein, bovine serum albumin and uses aluminum hydroxide as an adjuvant. The bovine serum albumin was selected because it has been widely tested for other purposes and has an established safety record in humans (Kantak *et al*., 2000). Furthermore, it has several chemical sites at exposed lysine residues that allowed relatively efficient succinyl conjugation with seven to eight morpine-6-succinyl molecules (Akbarzadeh *et al*., 1999; Kosten *et al*., 2000). Morphine-6-succinyl-bovine serum albumin has been shown to produce antibodies against morphine in animals. This vaccine generated catalytic antibodies against morphine and decreased self-administration of morphine in immunized rodents. The objectives of this study were to determine, first, whether changes in self-administration behavior would be systematically related to catalytic antibody level and, second, how the catalytic antibody affected the self-administration of different dose of morphine after active-immunization (Jertborn *et al*., 2001; Lerner and Tramontano, 1988). The morphine vaccine induced average serum catalytic antibody levels of 4.8 μg mL^{-1} and reduced the re-acquisition of self-administration behavior by 1 mg kg^{-1} morphine when serum catalytic antibody levels exceeded 4.8 μg mL^{-1} by using active immunization. Prior to initiating the trial, this therapeutic vaccine was tested in animals and showed no toxicity at several times proposed doses in humans (Landry, 1997). The animals toxicity testing included six group animals' studies 2 week long multiple dose rate study and six special toxicity studies in mice, balb.c, rat, guinea-pig, rabbit and hamster. This animal's study included histopathological examinations of organs, blood cells and serum catalytic antibody and biochemical changes. There were no signs of systemic toxicity, but there were local reactions at the injection sites due to the mechanical process of injection on daily basis. The mouse studies examined any toxicity from administering morphine to immunized animals and found lower levels of morphine induced mortality in the immunized compared to non-immunized animals, as expected (Gawin and Ellinwood, 1988). A second study showed that the vaccine did not produce any toxicity in animals (Svennerholm *et al*., 1983). Thus, animal studies showed no limiting toxicity. Nevertheless, expected adverse events may be similar to those observed with other subunit vaccines containing aluminum hydroxide as an adjuvant. Adverse events associated with aluminum adjuvant include erythema and subcutaneous nodules at the injection site. Previous studies with the bovine serum albumin carrier had not produced significant systemic adverse events (Svennerholm *et al*., 1983; Mclellan *et al*., 1992). However, both early (1-3) days and late (4-10 days) local reactions were observed by about half the subjects in the Svennerholm study after immunization (Cohen, 1997; Hal and Carter, 2004). Systemic gastrointestinal symptoms occurred in less than 10% of the subjects. The therapeutic morphine vaccine itself was not expected to have any morphine-like side effects that might produce psychoactive effects such as mania, because no free psychoactive drug components from morphine or active derivatives should be found in the circulation after administering therapeutic morphine vaccine for several reasons. First, the bovine serum albumin is covalently linked to the morpine-6-succinyl by a stable, amide linkage. Second, the amount of morpine-6-succinyl

contained in the vaccine, even if released by breaking the covalent bond, is about 1000 times lower than the typical human doses of morphine (e.g.,10 mg/dose) (Landry *et al*., 1993; Yang *et al*., 1996). Finally, no morphine metabolite at highest concentration in urine where it can be detected at levels down to 50 μg mL^{-1} was detected in urine samples.

Subjects and Methods for Clinical Trial of Morphine Vaccine

Study Design

This economic study was designed as a randomized, double-blind, placebo-controlled trial. Participants were outpatient volunteer addicts as this phase conducted to evaluate the safety and immunogenicity of Therapeutic Morphine Vaccine in humans in a cohort of abstinent morphine abusers who were outpatients for drug treatment program and then followed for 1 year after initial vaccination by which 95% of subjects were recovered when had completed the treatment program. In this study we evaluated local and systemic adverse events (Akbarzadeh *et al*., 2002, 1999).

Therapeutic Morphine Vaccine Formulation

The active component of the therapeutic morphine vaccine was morpine-6-succinyl linked to a carrier protein bovine serum albumin. This protein conjugate was adsorbed onto aluminum hydroxide gel as an adjuvant and suspended in Phosphate Buffered Saline (PBS) in single dose amount, in brown vials for deltoid intra-muscular injection. The vaccine was manufactured to strengths of 12.5, 100 and 600 μg mL^{-1}, a dose volume of 1.0 mL was administered. The placebo formulation consisted of the adjuvant and Phosphate Buffered Saline (PBS) but did not include the morphine-6-succinyl-bovine serum albumin component. The test material was provided in single dose use vials as a ready-to-use suspension stored at 2-8°C. The vials were brought to room temperature and shaken gently to ensure uniform suspension prior to administration. We did not give the carrier protein alone as the placebo in order to have maximum sensitivity for detecting any adverse effects from this carrier or the combined carrier and morpine-6-succinyl product. Using the carrier as the placebo might have raised our placebo group's adverse reaction rates, if the carrier itself was likely to produce adverse effects. Thus, our study provides a conservative estimate of the adverse effects of this therapeutic vaccine, although previous studies have not suggested adverse reactions to the bovine serum albumin carrier (Akbarzadeh *et al*., 1999; Kosten *et al*., 2000).

Selection of under Study Population for Clinical Trial of Morphine Vaccine

This economic outpatient study was conducted at outpatients form subjects (they were free and allowed to use morphine) treatment for former morphine dependent subjects where the expected length of study was at least 12 months. In order to qualify the study, a subject had to have been enrolled in this program for at least one month with documented abstinence from all illicit drugs on three times per week urine toxicologies. These observed urine toxicologies were continued three times weekly throughout the study. Baseline assessments of physical health included physical examination, electrocardiogram and laboratory blood studies. Screening exclusions involved major medical or psychiatric disorders, immunodeficiency including HIV infection and other medications including analgesics, antipyretics and immunomodulators (Svennerholm *et al*., 1983). A structured evaluation of each subject was done by a board certified psychiatrist using all available data sources including review of outside and the living program's medical records, which involved review of a 3 h intake evaluation by the Pasteur Institute facility and the Addiction Severity Index (ASI) (Mclellan *et al*., 1992). The ASI is a 45 min interview that covers seven major problem areas during the substance abuser's lifetime and previous 30 day. These problem areas are occupational, family,

medical, legal, psychological, drug and alcohol. After this extensive review and direct subject interview by the psychiatrist, subjects meeting Diagnostic and Statistical Manual of Mental Disorder (DSM-3R) criteria for any psychotic disorder, or lifetime major depressive disorder were excluded. Any current dysthymia or minor mood or anxiety disorder was an exclusion factor. DSM-3R criteria were also met for lifetime morphine dependence disorders; although due to lack of recent use subjects did not meet current dependence criteria for any substance at admission to the study (Cohen, 1997). The outcomes for this screening are presented in results section. All subjects signed a written informed consent form approved by the Iran national health committee. They were offered no financial inducement for their initial participation and they came back for follow-up interview at the 5, 7, 9, 11 and 12 months time points.

Safety Monitoring of Morphine Vaccine

The investigators monitored local and systemic adverse events occurring within 3 days of vaccination and subjects reported adverse events beyond this time point at subsequent clinic visits. Oral temperature, vital signs and inspection of the injection site were done at every day post injection for 3 days. Pyrexia after vaccination was defined as greater than 37.5°C. Injection site adverse events were classified into erythema, indurations, heat, edema, pain and tenderness. A physical examination was performed prior to each injection and on day 90. Any medications that were needed or any medical interventions done after vaccination were recorded. Routine biochemistry and hematology tests were performed on blood samples taken throughout the study on days 0, 30, 60 and 90. All treatments related adverse events that occurred in more than one subject for any dosage cohort was tabled (Table 1; 2-5 vaccination schedules). Three cohorts of 34 subjects, each were planned for enrollment, with 30 to receive, therapeutic morphine vaccine and four to receive placebo. Each successive group

Table 1: Number of subjects reporting treatment-related adverse events per treatment cohort [a,b]

		Therapeutic Morphine Vaccine		
	Placebo			
	0 μg mL^{-1}	12.5 μg mL^{-1}	100 μg mL^{-1}	600 μg mL^{-1}
Body system/costart term	(n = 12)	(n = 30)	(n = 30)	(n = 30)
Body as whole				
Elevated oral temperature	6 (50)	15 (50)	18 (60)	15 (50)
Headache	1 (8.3)	2 (6.6)	2 (6.6)	0 (0)
Dizziness	1 (8.3)	0 (0)	0 (0)	0 (0)
Somnolence	0 (0)	1 (3.3)	0 (0)	0 (0)
Injection sit reaction	2 (16.6)	0 (0)	1 (3.3)	1 (3.2)
Pain (left arm)	0 (0)	1 (3.3)	0 (0)	0 (0)
Ecchymosis	1 (8.3)	1 (3.3)	0 (0)	0 (0)
Pruritus	1 (8.3)	1 (3.3)	0 (0)	0 (0)
Laboratory test abnormal	0 (0)	0 (0)	1 (3.3)	1 (3.3)
Cardiovascular				
Hypertension	1 (8.3)	2 (6.6)	4 (13.2)	9 (30)
Tachycardia	2 (16.6)	4 (13.2)	7 (23.3)	6 (20)
Bradycardia	0 (0)	0 (0)	0 (0)	0 (0)
Digestive				
Nausea	0 (0)	1 (3.3)	0 (0)	0 (0)
Dyspepsia	0 (0)	0 (0)	0 (0)	0 (0)
Musculoskeletal				
Twitch	0 (0)	0 (0)	0 (0)	3 (10)
Arthralgia	1 (8.3)	0 (0)	0 (0)	0 (0)
Myalgia	0 (0)	1 (3.3)	0 (0)	0 (0)
Back pain	1 (8.3)	0 (0)	0 (0)	0 (0)
Hypertonia	0 (0)	0 (0)	1 (3.3)	0 (0)
Respiratory				
Pharyngitis	0 (0)	1 (3.3)	2 (6.6)	0 (0)

[a] Table detailing all treatment-related adverse events. Treatment-related is defined as possibly related, probably related or definitely related, [b] Values shown in the parenthesis are in percent (%)

was given a higher dose of therapeutic morphine vaccine: 12.5 μg mL^{-1} for cohort 1, 100 μg mL^{-1} for cohort 2 and 600 μg mL^{-1} for cohort 3. Each subject received a course of three 1 mL intra -muscular injections into the deltoid muscle at 0-30-60 days using the appropriate dose. Assignment to vaccine or placebo was randomized and all injections were double blind. Blood samples for antibody analysis were taken on days 0, 30, 60 and 90. During one year follow-up period, blood samples were taken at the 5, 7, 9, 11 and 12 months after initial vaccination. The placebo subjects were not followed up beyond day 90 for blood samples.

Serology and Detection of Catalytic Anti-morphine Antibody

The immunogenicity of the therapeutic morphine vaccine was assessed by measuring antibody levels specific for morphine by a direct ELISA method. Serum samples were taken as described above and frozen at -20°C until the time of assay. ELISA plates were coated with morphine-6-succinate coupled to Hen Egg Lysozyme (HEL), to ensure that the detected antibodies were specific for the hapten (morphine-6-succinate) and not for the carrier protein (bovine serum albumin). Three-fold serial dilutions, starting at 1:50 serum dilution, were made in phosphate buffered saline containing 0.05% tween 20 and 20 μg mL^{-1} Hen Egg Lysozyme. Samples were incubated on the ELISA plates overnight at 25°C. Specific IgG binding was detected with horseradish-peroxidase-labeled goat anti-human IgG antibody at 1:15,000 dilutions. The plates were developed with the substrate *o*-phenylenediamine hydrochloride (OPD) fixed with H_2SO_4 and the Optical Density (OD) was read at a wavelength of 490 nm. As a standard for antibody level determination, equal volumes from part of the day 90 serum samples from all 30 subjects whom were vaccinated with the lowest vaccine dose (12.5 μg mL^{-1} cohort 1) were pooled and the antibody level in this aggregated sample was determined. The OD level (reflecting amount of antibody) for this aggregated sample was arbitrarily defined as 100 units of anti-morphine antibody (Hal and Carter, 2004). The day 90 samples were chosen because this was when the peak antibody response was expected. The baseline or non-specific response was defined using the same anti-morphine assay procedure in 40 serum samples collected from untreated, non-psychiatric and non-substance abusing subjects in an unrelated study. In these 40 samples from normal subjects the mean OD value with±0.007 standard deviations was 0.125 at the 1:50 serum dilution. This OD value was 12.5% of the mean value for the day 90 samples from cohort 1, which had been defined as 100 units. Thus, any sample that has been presented in Table 2 in placebo group as

Table 2: Average of anti-morphine antibody levels generated by therapeutic morphine vaccine in three cohorts (1, 2 and 3) of addicted subjects whom were injected with 12.5, 100 and 600 μg mL^{-1} dose of morphine vaccine, respectively and placebo group

		Human IgG antibody to morphine (days)								
Screen No.	Vaccine									
12 subjects	Placebo	0	30	60	90	150	210	270	230	360
Sum	"	160.8	171.6	174	171.8	-	-	-	-	-
Average	"	13.4	14.3	14.5	14.2	-	-	-	-	-
SD±	"	0.23	0.12	0.15	0.62	-	-	-	-	-
30 subjects	12.5 μg mL^{-1} vaccine									
Sum	"	420	1515	2709	3015	5412	6106	1812	1524	1365
Average	"	13.4	50.5	90.3	100.5	80.4	70.2	60.4	50.8	45.5
SD±	"	0.46	1.93	2.42	3.41	4.63	3.83	3.75	3.65	2.87
30 subjects	100 μg mL^{-1} vaccine									
Sum	"	397	2259	3312	4524	3006	2712	2259	2112	1806
Average	"	13.3	75.3	110.4	150.8	100.2	90.4	75.3	70.4	60.2
SD±	"	0.96	5.45	9.45	5.05	9.59	9.17	11.3	7.79	12.85
30 subjects	600 μg mL^{-1} vaccine									
Sum	"	405	4524	7521	8259	6027	5262	3018	2415	2121
Average	"	13.5	150.8	250.7	275.3	200.9	175.4	100.6	80.5	70.7
SD±	"	0.64	5.57	7.03	11.27	16.96	9.77	10.12	6.83	11.85

Table 2 shows the human anti-morphine IgG expressed in arbitrary units described in the text, where the average serum value for 102 subjects at day 0 in all cohorts before vaccination, was assigned a value of 100 units and values of 13.4 represent no significant difference from a normal comparison group not exposed to therapeutic morphine vaccine. All 102 subjects received all three doses of either active vaccine or placebo and were supervised up to day 90th, but cohort 1-3 completed the study up to day 360th

13.4 represents the upper limit of the 95% confidence interval for the lowest sensitivity of this antibody assay and should be considered as representing no anti-morphine antibody present. Antibody responses to the therapeutic morphine vaccine itself, morphine-6-succinyl-bovine-serum-albomine, therapeutic morphine vaccine and to the carrier protein were also measured by ELISA method. The antibody levels were analyzed using repeated measures analysis of variance to compare the three doses of vaccine to placebo. By employing the statistical package for social sciences (SPSS) we conducted analyses out to day 90th and the mentioned analyzes were conducted on data obtained up to day 90th (Table 2).

Results

Demographics and Screening Results

We screened 200 subjects for this study and 98 failed to pass the screening procedure. 102 subjects in this study had a mean age of 25 years (19-40 years old) and all of them were male. Subject retention was acceptable for this type of population with 102 out of 200 subjects getting all three doses of placebo and morphine vaccine and completing the initial 3 months protocol. The subjects whom followed-up the study included 12 placebos, 30 in 12.5 μg mL^{-1} doses, 30 in 100 μg mL^{-1} dose and 30 in 600 μg mL^{-1} doses. In the year after the initial vaccination, 98 subjects completed the follow-up period.

Safety of Therapeutic Morphine Vaccine (TMV)

Therapeutic Morphine Vaccine (TMV) was well tolerated locally and systemically. Slight symptoms monitored at the injection site (local adverse events) were local pain, tenderness, indurations, heat, erythema and edema. In compare to other ten kinds of different vaccines (home-made and foreign-made) which are being used at the Pasteur Institute of Iran, the adverse events of therapeutic morphine vaccine were less than other ten kinds of vaccines. All reported injection site having adverse events following immunization were mild in severity and short-lived. The most frequent reported local adverse events were local pain and tenderness, reported in 98/102 subjects. There were few reports on indurations, heat, erythema and edema and 18/102 subjects reported 1 or more of these symptoms. There was no pattern of incidence according to dose level or vaccination sequence for any of the reported local adverse events. The most frequent treatment-related systemic adverse events were tachycardia, elevated temperature, hypertension and headache in the placebo group, together with tachycardia, elevated temperature, hypertension, headache pharyngitis, twitching and nausea for morphine vaccine (all dosage groups). Table 1 lists treatment-related adverse events, which were in the opinion of the investigators, possibly, probably, or definitely related to medication during days 1-90. The Systemic Adverse Events (SAE) were considered severe in intensity but not related to medication. There were no significant changes in vital sign measurements other than a possible correlation between dose and elevation of oral temperature. The greatest mean increase in oral temperature (+ 0.7°C) was seen at the highest dose. However, temperature elevation was seen in some subjects in all groups (including the placebo group) after each vaccination. The frequency of temperature elevation above (37.2°C) ranged from no reports in the 12.5 μg mL^{-1} dose group after the third vaccination to 12/30 subjects in the 100 μg mL^{-1} dose group after the first vaccination. The highest recorded temperature was (37.9°C) 64 h after the second injection, elevated from a pre-dose figure, on the day of vaccination, of (37.1°C) for two subject in the 600 μg mL^{-1} dose group. The only treatment-related adverse event following immunization suggesting a dose relation was muscle twitch. This occurred in subjects in the highest dose group. In each case twitching was in the arm into which the vaccine had been administered. All such events were mild in severity and resolved within the first 48 h. Future studies will carefully monitor the muscle twitches as well as other local and systemic adverse events reported in this study. In 90 subjects whom followed-up the study to 1 year, there were no reports of adverse events after the initial 3 months study period; this included those subjects whom stayed in the study program for the full year.

Therapeutic Morphine Vaccine (TMV) Elicit Specific Serum Anti-morphine Antibodies

Morphine vaccine induced morphine-specific antibody in all three cohorts. Table 2 shows the average anti-morphine antibody responses for 90 subjects whom received all three therapeutic morphine vaccine injections and remained in the study through day 90 and for 12 placebo subjects. Data has been presented for the average of each cohort at each time point. The first four time points represent the 90 non-placebo subjects. The last five time points represent only those 90 subjects whom participated in the follow-up phase of the study, as shown in Table 2. Anti-morphine antibody responses above background were not detected prior to vaccination. The first clearly detectable IgG anti-morphine antibody appeared by ELISA method after the first injection of 100 and 600 μg mL^{-1} and second injection of 12.5 μg mL^{-1} doses and reached to their peak in 3 months and did not decline to baseline after one year. All three doses produced a robust antibody response after the second vaccination and the response increased after the third vaccination. There was substantial individual to individual variability in the magnitude of the antibody response within each cohort. By using a repeated measures ANOVA that included the placebo group and analyzed to day 90, significant effects were shown for time ($F = 100.8$; $df = 3, 4.4$; $p<0.001$), dose ($F = 14367.8$; $df = 3,98$; $p<0.001$) and the interaction of time by dose ($F = 1.9$; $df = 3, 12$; $p<0.187$). Excluding the placebo cohort still led to a significant time by dose interaction ($F = 3.4$; $df = 3, 8$; $p<0.075$). Finally, in the statistical contrast between the 600 μg mL^{-1} cohort and the other two therapeutic morphine vaccine cohorts, the dose effect was significant ($F = 1817.0$; $df = 1, 88$; $p<0.001$). Thus, less significant difference in the response was seen between cohort 1 and 2, but the highest dose (600 μg mL^{-1}, cohort 3) induced a significantly higher antibody response. In 90 subjects whom followed up the study to 1 year, the antibody levels did not decline to baseline for all three cohorts by 1 year, as shown in Table 2. The rate of decline in antibody levels was comparable in all three cohorts. Although these data include only 90 of the 102 vaccinated patients whom completed 360 days, examination of the individual responses in Table 2 reveals that the rate of decline in antibody response was fairly consistent for all subjects in the study.

Discussion

The therapeutic morphine vaccine was well tolerated when administrated as a course of three injections of 12.5, 100 and 600 μg mL^{-1} given at monthly intervals to abstinent morphine abusers. No serious vaccine- related adverse events were reported during the 3 months study period or during the 12 months follow-up. When most subjects had left the immunotherapy programs, 95% of them were recovered. Minor adverse events included small temperature elevations in about one-third of the subjects, mild pain and tenderness at injection site and muscle twitch at the highest dose, as well as a range of minor systemic reactions. Twitching was reported only at the highest dose; except for this, the frequency of reports was comparable in all groups including placebo.

We studied any events of twitching in different stages of vaccination, with 100 μg mL^{-1} dose of morphine vaccine. With depend on the pattern of incidence, we considered the changes and corrected our procedure. We found that the best dose of morphine vaccine for injection in human is 100 μg mL^{-1}. No other adverse events had a correlation with vaccination dose and there were no correlations of adverse events with vaccination sequence. Therapeutic morphine vaccine induced morphine-specific antibody in all vaccinated subjects. This antigen-specific IgG was detectable after the second vaccination and increased in all groups after the third vaccination at day 90. There was a statistically significant higher antibody response in cohort 3 than other two cohorts ($p<0.001$), but there was difference in the antibody responses between group 1 and 2 at day 90th. Thus the antibody levels persisted beyond 1 year for all of the dosage cohorts. These clinical trial results are promising, warranting further voluntarily and generally vaccination of addicted persons with the therapeutic

morphine vaccine approach. The levels of antibody induced in this study were measured by ELISA method (Kantak *et al*., 2000; Landry *et al*., 1993). In other study, by investigating in 1240 addicts, we found that the ultimate dose of morphine vaccine for immunotherapy and induction of acceptable amount of morphine antibody is three 100 μg mL doses of morphine vaccine. This therapeutic vaccine will be most effective for relapse prevention in morphine abusers who are motivated to preserve their abstinence, since it is likely that subjects will be able to overwhelm the anti-morphine antibody by using sufficient amount of morphine. However, the vaccine may be able to prevent a morphine slip from turning into full-scale binge and relapse to dependence. When a small amount of the abused substance is used, it stimulates craving for more of that substance leading to a morphine binge for morphine users (Yang *et al*., 1996). The therapeutic morphine vaccine is a most effective in reducing the priming effect of using small to modest amounts of morphine. The potential target population of morphine abusers needing this type of relapse prevention probably encompasses the majority of the 3,000,000 morphine abusers seeking treatment annually, but this number could increase to encompass more of the three million abusers, if an effective medication like this method, would not be available. Only outpatient studies will be able to evaluate this treatment potential, although morphine administration studies in humans may also illuminate this mechanism of action in reducing craving induced by modest single doses of morphine that characterize a typical slip in newly abstinent morphine abusers.

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