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Immunotherapy of 347 Volunteer Outpatient Morphine Addicts by Human Therapeutic Morphine Vaccine in Kermanshah Province of Iran

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Abstract: The effective constituent of human therapeutic morphine vaccine is morphine-6-succinate-BSA which would be produced by mixed anhydride method. By injection of 3 doses of vaccine at the interval of 0-30-60 days, humoral immunity would be caused in addicts. In this study 347 morphine addicted volunteers were vaccinated with therapeutic morphine vaccine according to WHO and national vaccination protocol. The variables were doses of vaccine, concentration of anti-morphine antibody, total protein and gamaglobuline. Volunteers were bled and then injected at the interval of 0-30-60 days. All subjects were bled at day 90 and after 1 year, 10% of them were bled randomly. Total protein and gamaglobuline were determined by serum electrophoresis and anti-morphine antibody level was estimated by ELISA. Considered variables were directly correlated with number of injections that were detected on 30 days after the first injection reaching their peak by three months after first injections and were not declined to the baseline by 1 year. All subjects were followed up and monitored for 1 year. The vaccine was well tolerated by addicted volunteers and had no serious drug-related adverse events. Only 1% at the first dose experienced brief post injection twitching and all subjects were immunized.

Key words: Clinical trial, volunteer addicts, vaccination, morphine vaccine

INTRODUCTION

In 2002, the population of Iran was 67000000 which 3,000,000 of them (4.4776%) were morphine abusers. The total number of drug users in the world is now estimated to be around 200 million people, equivalent to about 5% of the global population aged 15-64. (World Drug Report, 2006). The research carried out by different groups all over the world, could not tackle this social obstacle and day by day the numbers are increasing. Historically, the abuse of opium started 4000 years B.C by man i.e., Sumerians for the first time then Egyptians and Persians used opium. According to WHO report, 3.13% of the world population is suffering by being addicted to narcotic drugs and the number is going up day by day. The overcome of this obstacle is of prime importance and introduction of a therapeutic morphine vaccine that immunizes the susceptible population against morphine will protect such population (Akbarzadeh *et al.*, 2001a). In this research, 347 persons whom were addicted to morphine were given the shots of morphine vaccine and its reactions in those people were studied. The idea of immunotherapy by humane Therapeutic Morphine Vaccine, goes back to early 80, getting the idea by battling war gases like nitrogen mustered, thio- mustered through vaccines against them. Vaccines made against such gases somehow immunized the people against nitrogen and thio mustered gases. This was led to the production of new generation of vaccines i.e., synthetic

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vaccines against such gases. In 1990, production of antibody against morphine was started in our laboratory. In 1994, a rapid diagnostic kit to detect morphine in urine was introduced (Akbarzadeh *et al.*, 2001b). In 1999, the morphine vaccine was produced in our laboratory and many morphine abusers were vaccinated by such vaccine (Akbarzadeh *et al.*, 2003, 2007). This vaccine is produced and controlled according to WHO protocols for vaccines production and quality control. This type of immunotherapy will help the morphine abusers to stop morphine after being vaccinated by vaccine. They neither show any desire to restart morphine abuse nor encounter any life threatening disorders. The psychological addiction in those who are consuming morphine can be disabling and may lead to relapse. The therapeutic morphine vaccine provides a unique approach rather than pharmacotherapy employed to treat the morphine addicted individuals (Akbarzadeh *et al.*, 2002). The mechanism of the action of morphine vaccine can be explained as follows: after the immunization of the addicted individual by such vaccine, if an addicted person consumes morphine, 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 (Akbarzadeh *et al.*, 1999). Furthermore, a therapeutic morphine vaccine based on active immunization has the potential to provide long lasting efficacy in order to prevent relapse with less problem and compliances in individuals who desire to give up their addiction to morphine. This type of vaccine therapy is based on humoral immunity. After administration, it brings about long term immunity which in turn prevents the relapse and does not produce any psychological disorders and break syndrome in those who are willing to give up their addiction to morphine (Akbarzadeh *et al.*, 2007; Berrettini and Lerman, 2005).

MATERIALS AND METHODS

This research was conducted to study the effects of immunotherapy of 347 volunteer outpatient morphine abusers with morphine vaccine in Kermanshah Province of Iran during 1999-2000. The average age of volunteers was 29.7 (15-62 years old). One hundred and sixty parameters of WHO and Iran national protocols were considered for synthesis of therapeutic morphine vaccine. The effective constituent of human therapeutic morphine vaccine is morphine-6-succinate-BSA which would be produced by mixed anhydride method (Table 1).

Human Studies

The population under study immunized by morphine vaccine has been evaluated through considering 160 parameters included in the evaluation form prepared according to WHO and national protocols. Three hundreds and forty seven morphine addicted volunteers with average age of 29.7 (15-62 years old) including 319 males and 28 females were immunized with morphine vaccine. The variables were vaccine dose, concentration of anti morphine antibody, total protein and gamma globulin of the blood system (Akbarzadeh *et al.*, 2007).

Table 1: Component, amount and function which consist of one dose of manufactured morphine vaccine

Components	Amount	Function of components in vaccine
Morphine-6-succinate-BSA	50 µg	Antigen
Aluminum hydroxide (Al ³⁺)	0.5 mg	Adjuvant
Sodium chloride	8 mg	Buffer
Dibasic sodium phosphate anhydrous	1.12 mg	Buffer
Monobasic sodium phosphate monohydrate	1.1 mg	Buffer
Water for injection Qs	1 mL	Solvent

Table 2: Changes in mean values of anti-morphine antibody, total protein and gammaglobuline in 347 addicted volunteers in 360 days after three doses of injection

Variables	Days of bleeding				
	0	30	60	90	360
Days of vaccination	0.000	30.000	60.000	-	-
Mean value of total protein (g dL ⁻¹)	7.419	7.587	7.726	7.745	7.650
Mean value of gammaglobuline (g dL ⁻¹)	1.214	1.252	1.361	1.391	1.281
Mean value of anti-morphine antibody (g dL ⁻¹)	0.910	0.939	1.020	0.968	0.096

Selection of Population under Study

The evaluation forms for addicted individuals as outpatients were prepared according to WHO and national vaccination protocols and were filled out (Akbarzadeh *et al.*, 2007; Mihältan, 2007).

Side Effect Studies of Morphine Vaccine

Detrimental effects of the vaccine after each vaccination of individual under study were not observed. After each shot, the subjects were examined for body temperature, detriment in site of injection such as swelling, skin redness in the form of erythma, indurations, edema and allergy which none of these effects were observed (Tobin *et al.*, 2005; Keyler *et al.*, 2005).

Immunological Evaluation

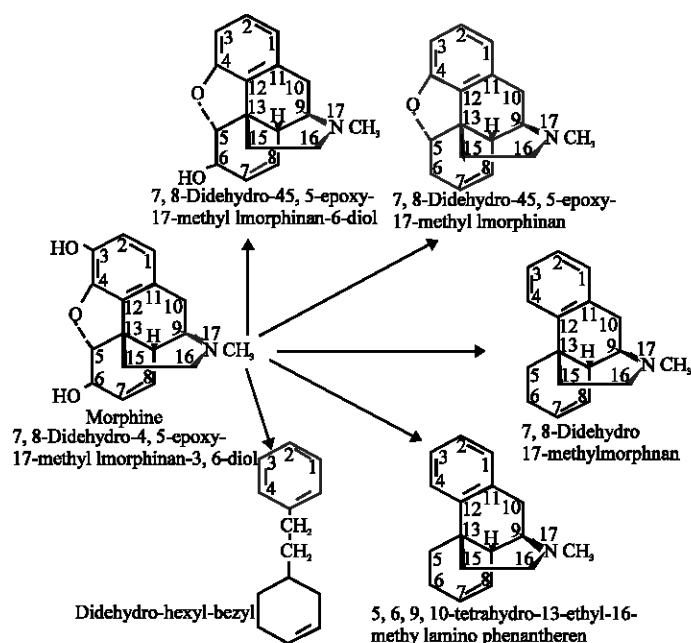
Immunity of the vaccine was evaluated by measuring the total protein, gamma globulin and anti-morphine antibody in serum of the vaccinated volunteers by electrophoresis and by direct ELISA method. Sampling of the volunteers was performed up to the day 90th and only in 10% of them continued up to day 360th (Table 2) (Akbarzadeh *et al.*, 2007; Mihältan, 2007).

Morphine Catabolism in Circulatory System by Catalytic Anti-Morphine Antibody and Prevention of Morphine to Get into Brain

The anti-morphine catalytic antibody, which would be produced against morphine in circulatory system would block and change the administered morphine molecules into 7,8-didehydro-4,5-epoxy-17-methylmorphinan-6-diol/7,8-didehydro-4,5-epoxy-17-methylmorphinan/7,8-didehydro-17-methylmorphinan and 5,6,10-tetrahydro-13-ethyl-16-methylamino phenantheren, respectively and finally into didehydro-hexyl-benzyl, which is a non-analgesic molecule. After immunization with morphine vaccine and production of anti-morphine catalytic antibody, the changing rate is 80-100 morphine molecules per second. The mechanism of the action of morphine vaccine can be explained as follows: after the immunization of the addicted individual by such vaccine, if an addicted person consumes morphine, 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 (Akbarzadeh *et al.*, 2007, 1999). Furthermore, a therapeutic morphine vaccine based on active immunization has the potential to provide long lasting efficacy in order to prevent relapse with less problem and compliances in individuals who desire to give up their addiction to morphine.

Morphine Catabolism in Circulatory System by Catalytic Anti-Morphine Antibody and Prevention of Morphine to Get into Brain in the Circulatory System of Morphine Addict

The following scheme is the result of *in vitro* morphine hydrolysis with anti-morphine antibody at 37°C and detection and determination of chemical groups by colorimetric, IR and mass spectroscopy methods.



RESULTS AND DISCUSSION

Morphine vaccine has been well tolerated systematically. Signs like local pain, allergy, rigidity of tissues, fever, inflammation and slight edema on the skin were less observed in the volunteers injected with morphine vaccine as compared to the other 10 kinds of vaccines like B.C.G., Polio, M.M.R and etc being injected at the department of vaccination of Pasteur Institute of Iran, Tehran, thereby showing less unfavorable effects. Morphine vaccine causes the production of specific morphine antibodies resulting in raise of total amount of protein, gamma globulin and anti-morphine antibody (Table 2). Table 2 shows the mean values of the total protein, gammaglobuline and anti-morphine antibody in 5 steps of volunteers bleeding. Decrease in the amount of antibody, total protein and gammaglobuline in 12 months was natural and did not reach the base line. Responses to increase in the amount of anti-morphine antibody, total protein and gammaglobuline above the base line were followed up after first vaccination. The first detectable increase in the amount of anti-morphine antibody, total protein and gammaglobuline was observed 30 days after first vaccination. After second vaccination strong responses of anti-morphine antibody, total protein and gammaglobuline were observed and on third vaccination the above elements were increased. The amount and intensity of the anti-morphine antibody, total protein and gamma globulin response were varied from person to person.

There are no reports on morphine vaccine other than ours, so there were no references on this issue to refer (Akbarzadeh *et al.*, 2002). Morphine vaccine gives better result as compared to cigarette and cocaine vaccines (Daniel, 2005; Vocci and Chiang, 2001). By analyzing the data obtained from the forms of the immunized addicted volunteers through Access program (Table 3); it indicates the number of people whom were treated. It is essential to bring into the notice that in this research the sisters and brothers of the addicted people were not considered and if they were considered, the horror of the addiction would appear. Estimation of annual prevalence of drug abuse at the global level in the late 1990s and 2001-2003 shown in Fig. 1.

Table 3: Extent of drug abuse (annual prevalence*) 2001-2003

Comparison	All illicit drugs	Cannabis	Amphetamine-type stimulants				Opiates	Heroin
			Amphetamines	Ecstasy	Cocaine			
Million people	185.0	146.2	29.6	8.3	13.3	15.2	9.2	
In compare to								
Global population (%)	3.0	2.3	0.5	0.1	0.2	0.2	0.2	
Global population	4.7	3.7	0.7	0.2	0.3	0.4	0.2	
Age (15-64 years old) (%)								

*Annual prevalence: a measure of the number/percentage of people who have consumed an illicit drug at least once in the 12 month- preceding the assessment

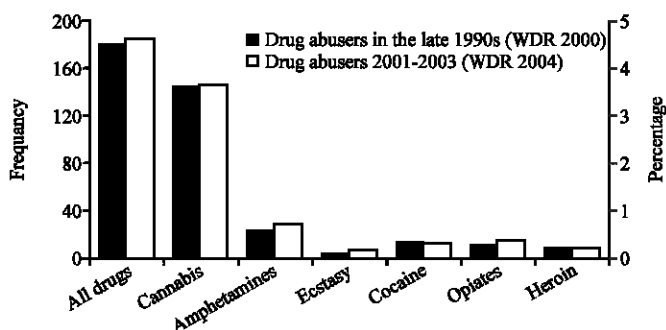


Fig. 1: Estimation of annual prevalence of drug abuse at the global level in the late 1990s and 2001-2003. Source: UNODC Annual report, questionnaire data, national report

CONCLUSION

In this study, the levels of anti-morphine antibodies, total protein and gamma-globuline were declined but did not reach the primary level (base line) in one year. To maintain such antibody level, booster dose is recommended after one year. It seems the levels of antibodies, total protein and gammaglobuline started to decline after about four months (two months after last dose) (Table 1) and this decline is acceptable. The results obtained through clinical studies hopefully guarantee the vaccination of the morphine addicted persons. Likewise, high level of morphine antibody descends the desire of morphine addicts to restart morphine consumption. Morphine vaccine prevents the vaccinated morphine addicts from restarting the consumption of morphine because the antibody destroys the in taken morphine. The vaccine also prevents the progress of addiction and relapse. When less amount of such narcotic is consumed by a person for the first time, it causes stimulation and desire to reuse the drug which results in more consumption leading to addiction. This vaccine highly affects the primary effects of low or medium dose of morphine consumption. The number of addicts who need to be prevented from restarting the consumption of drug is high therefore, they seek kind of treatment with positive outcome but so far such results have not been achieved and this type of vaccination can yield suitable solution to the drug abusers' problem.

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