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Safety of Human Therapeutic Morphine Vaccine Employing Lohmann Specific Pathogen Free Eggs

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Abstract: One of the sensitive and standard tests to control the safety of a vaccine is the inoculation of such vaccine to the air pocket of Lohmann specific pathogen free eggs. The aim of this study is to control the safety of morphine vaccine. This study reveals the safety of morphine vaccine by employing Lohmann specific pathogen free embryo eggs. The changeable parameters in this test were: weight of eggs, safety of eggs embryo, vaccine concentration, normal saline and temperature of the incubator. To study, the safety of morphine vaccine, we used 30 eggs (after controlling the safety of eggs and their embryos) which were divided into two groups of control (15 eggs) and test (15 eggs). After weighing the eggs, the eggs under experiment were inoculated with morphine vaccine and the control group was inoculated with physiological solution. Both groups were incubated and weight of the eggs and chickens were determined accordingly. The eggs of each group were controlled by their weights showing healthy, normal growth and evolution. The comparison between the weights of control and experimental groups did not show any significant changes. Exactly growth and evolution of each group were found equally to be balancing for three weeks after injection. Finally all eggs were observed to be safe, alive and in evolutionary form. By comparing the growth and evolution amongst each egg in the group under experiment, after injection, the eggs did not show any adverse reaction after inoculation with therapeutic human morphine vaccine.

Key words: Safety, human morphine vaccine, inoculation, air pocket, Lohmann specific pathogen free eggs

INTRODUCTION

Fortunately nowadays, mankind is aware of addiction and drugs problem in the world. By attention to the United Nation Office on Drugs and Crime report (World Drug Reports) 2004, about 200 million people or 5% of the world's population aged 15-64, have used drugs at least once in the last 12 months (UNODC, 2004). This figure is 15 million people higher than last year's estimate but remains significantly lower than the number of persons using licit psychoactive substances (about 30% of the general adult population use tobacco and about half use alcohol). The number of cannabis users worldwide is now close to 160 million people or 4% of the population aged 15-64. Estimates of the number of Amphetamine-type stimulants (ATS) users-26 million people using amphetamines and 8 million using ecstasy-are slightly lower than those of last year's World Drug Report (WDR), reflecting declines of methamphetamine use in South-East Asia (notably Thailand) and of ecstasy use in North America (notably in the USA). The number of opiate users is estimated to have risen slightly to about 16 million people (11 million of which abuse heroin); mainly reflecting increasing levels of opiate abuse in Asia. No significant changes were observed in most other parts of the world. The number of cocaine users-close to 14 million people-rose slightly. Unsurprisingly, the main problem of drugs at the global level continue to be the opiates (notably heroin) followed by cocaine. For most of Europe and Asia, opiates continued to be the main drugs of problem, accounting for 62% of all treatment demand in 2003/04. In South-America, drug related treatment demand continued to be mainly linked to the abuse of cocaine (59% of all treatment demand). In Africa, the bulk of all treatment demand-as in the past-is linked to cannabis (64%). 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, 1998; Akbarzadeh and Farahmand, 2001).

The idea of immunotherapy by human therapeutic morphine vaccine, goes back to early 80, getting the idea

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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 vaccines against those 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 in Pilot Biotechnology Department-Pasteur Institute of Iran (Akbarzadeh *et al*., 2001). The morphine vaccine was produced in our laboratory and many morphine abusers were vaccinated by such vaccine (Akbarzadeh *et al*., 2003, 2007a). This vaccine is produced and controlled according to WHO protocols for vaccines production and controlled qualitatively. 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 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 he/she 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*., 2007b). 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 humeral 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*., 1999).

Recently, immunotherapy of 347 volunteer morphine addicts by human therapeutic morphine vaccine was conducted in Kermanshah province of Iran (Akbarzadeh *et al*., 2009). One of the sensitive, careful, qualified and accepted tests to control biological substances (vaccines) qualitatively (particularly human therapeutic morphine vaccine), is inoculation of biological substances (morphine vaccine) into air pocket of Lohmann specific pathogen free eggs.

MATERIALS AND METHODS

This study is a safety testing of morphine vaccine which is one of the many kinds of WHO and national protocols used in the quality control of vaccines and is a part of study and production of morphine vaccine project conducted at Pilot Biotechnology Department of Pasteur Institute of Iran since 1994. We studied the safety of morphine vaccine in the Lohmann specific pathogen free, safe embryo eggs (German Co.). The mentioned eggs which were produced in Lohmann (German Co.) were obtained from Razi Vaccine and Serum Research Institute. In all steps, throughout the work, level II of bio-safety precautions must be considered. The handling and the storage of eggs of embryo are simple. The eggs embryo cells are highly sensitive, perfectly natural and the outside agents couldn't affect the growth of embryo and thus not having effect on the control of the vaccine safety. All eggs were checked for existence of live embryo by laying eggs on two kinds of Candler and locating of embryo and air pocket (Fig. 1a, b). In each step 30 eggs were arranged on the eggs shelf, divided in two experimental and control groups, each group containing 15 eggs and transferred to the candling room. All eggs were checked for breakage or contamination on the shell. The pocket area was detected and marked by pencil. The area of embryo and injection marked on the eggs shell. On the shell of eggs, the numbers and the kind of substance for inoculation were noted (Fig. 2).

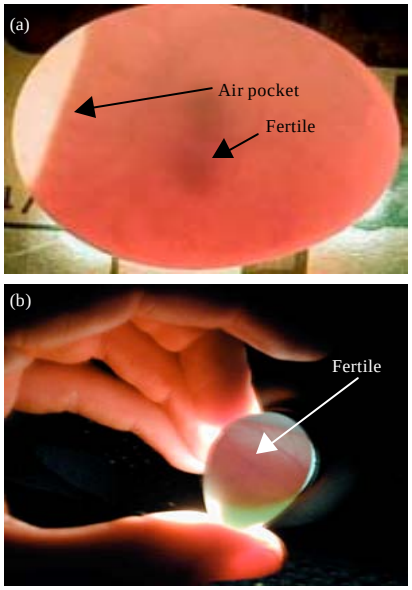

Fig. 1: (a, b) Checking of existence of live embryo by laying eggs on two kinds of Candler and locating of embryo and air pocket

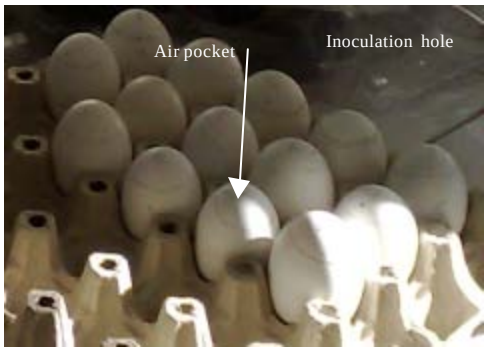

Fig. 2: The area of inoculation is air pocket

Compounds of morphine vaccine and inoculation: Each dose of morphine vaccine contains 0.5 mg of aluminum hydroxide, 8 mg of sodium hydroxide, 1.12 mg di-sodium hydrogen phosphate and 1.1 mg of sodium di-hydrogen phosphate monohydrate and 50 μg mL^{-1} of morphine-6-succinate-bovine serum albumin in 1 mL of water for injection.

Methods of morphine vaccine inoculation in fertile eggs: The eggs were procured freshly for this study. The age of eggs and the area of inoculation, with reference to the kind of vaccines are different. We employed fresh embryonic eggs inoculated with therapeutic human morphine vaccine. Of course, the method and the area of inoculation are correspondent to the age of eggs. If the inoculation site is Allan tonic area, the age of eggs should be between 10-12 days old. The site of embryo and the inoculation were marked on the eggs shell. The numbers and the kind of substances to be inoculated into the air pocket were written on the eggs shell (Fig. 2).

Inoculation of vaccine into the embryonated eggs and incubation of the eggs: The eggs were candled, weighed out in order to check the embryos' safety and arranged on egg shelf (Fig. 2 step a). They were transferred to laminar air flow cabinet. The shells of the eggs were disinfected by 70% alcohol (James *et al*., 1995; Katz and Webster, 1989). As soon as the eggs shells were dried, a hole was made through sterile needle on air pocket of eggs (Murphy and Strunk, 1985; Nolan *et al*., 2003) to inoculate one dose of morphine vaccine. Fifteen eggs were inoculated in such a manner under experiment group. The same procedure was followed for the other group termed as control but they were inoculated with normal saline. The holes on the eggs shell were sealed by molten solid paraffin (Murphy and Strunk, 1985; Kemp *et al*., 1990). After sealing the hole by molten paraffin, all eggs were weighed by using digital balance (± 0.01 g accuracy) and

Fig. 3: Sample chickens of experimental and control groups. (a) Samples of chicken of control group and (b) Samples of chicken of experimental group

incubated in incubator, to check probable contamination and personnel errors by candling after 24 h (Greenberg and Birx, 1988; Fasano *et al*., 1992). At this time, eggs containing dead embryos must be eliminated. The temperature of incubator was set at 37°C, 55±5% humidity and the best hatching ventilation results were obtained with normal atmospheric air, which usually contains 20-21% oxygen. The incubator was checked twice per day. After two and seven day of inoculation the eggs were weighed and on 20 days of inoculation the incubator was checked hourly. On 21 days after inoculation, chickens (Fig. 3a, b) were controlled hourly by visual observation (Kemp *et al*., 1990; Fasano *et al*., 1992). In final check, chickens were weighed and extinct eggs which did not hatch were observed (Khakoo and Lack, 2000). Three eggs from control and two eggs from experimental groups did not hatch.

RESULTS

One of the live and sensitive media for controlling the effects of biological materials is the employment of fertile eggs. The fertile eggs as media in comparison to other

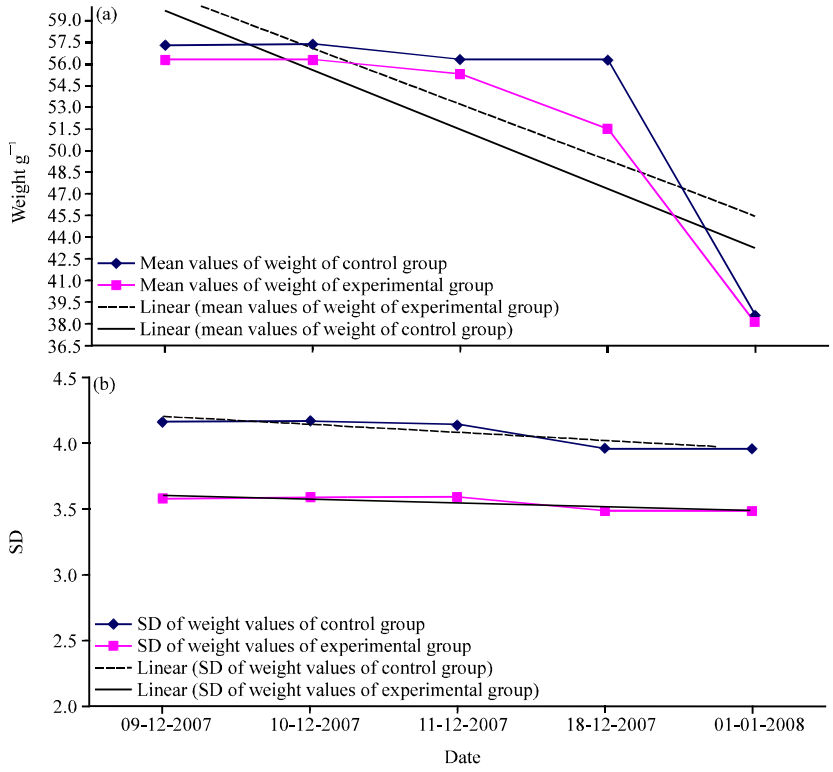

Fig. 4: (a) Mean values and (b) SD of weight of 15 eggs and chickens of experimental and control groups' eggs. It shows that through 23 days of study, growth and weight of chickens are parallel

media like cellular media are considered to be superior. Due to Calc shell content of fertile eggs and being closed media the entrance of contaminants in to them are impossible. The fertile medium in egg has different kinds of highly sensitive cells that may be suitable target to study the side effects of under trial biological materials. This type of method reveals the safety, sensitivity and adverse reaction which might be caused by the vaccines. If inoculated vaccine into the fertile causes any un-natural effect, it is considered unsafe and if it does not have any adverse reaction on the cells, it is safe. By considering the mean weight value obtained in two groups of eggs and chickens through 23 days, their standard deviation and curve comparison of mean weight values showed changes in the values to be parallel and linear. The parallelity and linearity change in mean value of the weights of two groups and standard deviation indicating three eggs were not hatched (20%) in control group and two eggs were not hatched in experimental group (13.3%) revealed that the morphine vaccine under study can be considered to be safe. Figure 4a shows the mean values of weight of 15 eggs and chickens of experimental and control groups' eggs. It shows that through 23 days of study, growth and weight of chickens are parallel. Figure 4b shows the standard deviation of weight values of 15 eggs and chickens under experimental and control groups. It shows that through 23 days of study, standard deviation of growth and weight of chickens are parallel.

DISCUSSION

There are number of studies carried out by various investigators who successfully showed the safe administration of measles vaccine to children allergic to egg (James *et al*., 1995), efficacy of inactivated influenza viral vaccine (virus H3N2) grown in mammalian cells or emberyonated egg (Katz and Webster, 1989), influenza vaccine in asthmatic children hypersensitive to egg protein (Murphy and Strunk, 1985), immunogenicity of live-attenuated influenza vaccine that was blended and filled at two manufacturing facilities (Nolan *et al*., 2003), mumps-measles-rubella vaccine which was safely administered to children allergic to egg (Greenberg and Birx, 1988), the allergic reaction caused by measles vaccine in patients hypersensitive to egg protein (Herman *et al*., 1983) and measles immunization of children with clinical reaction to egg protein has also been shown (Kemp *et al*., 1990). Egg hypersensitive and adverse reactions to measles, mumps and rubella vaccine were studied by Kemp A (Fasano *et al*., 1992). There are other

reports indicating recommendation of immunization by measles-mumps-rubella vaccine in children allergic to egg (Khakoo and Lack, 2000). Vandebriel *et al.* (2007) studied the effect of diphtheria-tetanus-acellular pertussis vaccine on immune responses in mouse local lymph node and lung allergic models. By comparison of natural growth and evolution of all fertile to chickens, in each egg groups after inoculation of morphine vaccine, they did not show any adverse reaction in response to human therapeutic morphine vaccine so it did not show any adverse event in all embryos cells too. As eggs embryos are highly sensitive cells and did not show any side effects and adverse reactions when inoculated with morphine vaccine, indicating that human therapeutic morphine vaccine is perfectly safe vaccine. During the last decades of research and production, the quality control of morphine vaccine in our laboratory was performed in parallel with Diphtheria, Tetanus, Pertussis, Mumps, Anthrax, vaccines in Lohmann specific pathogen free eggs and as comparing our work with number of studies of above mentioned investigators, we found that morphine vaccine is perfectly safe.

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