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Short Paper

Comparative study of antibody titers produced against two BHK rabies vaccines in field trial experimental condition in dogs by RFFIT

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Summary

Rabies is acutely fatal encephalitis caused by a neurotropic virus. This virus belongs to the family of Rhabdoviridae and genus of Lyssavirus. The virus is almost always transmitted to human through infected mammalian saliva. Rabies is inoculated to a wound by an infected animal bite. Since infection is established in the CNS, the outcome is almost always fatal. According to the outstanding role of vaccination against rabies in animals, as well as post-exposure treatment regimen in human, production of cell-cultured rabies vaccine is the most common interest of researchers. Two BHK-rabies vaccines, one made in the Pasteur Institute of Iran and two in Schering-Plough Animal Health of Madrid, Spain have been tested on 12 dogs divided into two groups. Sera were taken monthly over 18 months. To evaluate the titer of the rabies-neutralizing antibody, these sera were analyzed by the rapid fluorescence focus inhibition test (RFFIT) in the end of each month. Both study groups showed a sufficient immunogenic response without any significant difference at least one year after first vaccination. With respect to the effective immunization of rabies vaccines, their annual injections would be sufficient. According to the results, at the end of the study (18th month) serum titer in only one dog (No. 7) was less than protective level. Two animals in group one (No. 2 and No. 5) also had serum titers less than protective level. Mean of post vaccination antibody titer were not different in either groups (P=0.35).

Key words: Antibody titer, BHK rabies vaccine, Rapid fluorescent focus inhibition test

Introduction

Rabies is a zoonotic viral disease which infects domestic and wild animals. It is transmitted to other animals and humans through close contact with saliva from infected animals (i.e. bites, scratches, licks on broken skin and mucous membranes). Once symptoms of the disease develop, rabies is fatal to both animals and humans (Rad, 2009). Approximately 55000 people die from rabies each year (WHO, 2009). The vast majority of these deaths occur in Asia and Africa, and children are at particular risk. Annually, more than 10 million people, mostly in Asia, receive post-exposure vaccination against rabies (Rezaeinasab *et al*., 2007). There are two types of vaccines to protect humans against rabies: one, nerve tissue and the other, cell culture vaccines. The nerve tissue vaccines cause more reactions subsequent to administration and

are less potent than cell culture vaccines. WHO recommends replacement of nerve tissue vaccines with the more efficacious, safer vaccines developed through cell culture. It also advises that cell culture vaccines that have been specifically authorized for intradermal immunization represent an acceptable alternative to standard administration by the intramuscular route (WHO, 2009).

Administration through the intradermal route should be considered in settings where cell culture vaccines are unaffordable and/or in short supply. Periodic booster injections of rabies vaccine for persons whose occupation puts them at continuous or frequent risk of rabies exposure are advised (WHO, 2009). Human rabies has been eradicated in some developed countries, but it is still present in many others, including those countries in Southeast Asia (WHO, 2009). According to the outstanding role of vaccination to prevent rabies in animals as well as post exposure regimen in human beings, the production of new vaccines is one of the most favorite interests of researchers. This favored vaccine mostly makes a durable, safe, potent, and considerable immunological response in human and animals (Servat, 2006). One of these rabies vaccines is baby hamster kidney (BHK) vaccine, made in the Pasteur Institute of Iran (Fayaz *et al*., 1997) and Schering-Plough Animal Health, Madrid, Spain. This field trial experiment was designed to compare the function, competence, and level of mean antibody titers as well as the immunological response duration in dogs.

Materials and Methods

Admission and isolation of dogs

This study was performed in two groups of dogs, six healthy 3–4-month-olds without antibody titer against rabies in each. These dogs were under supervision of professional veterinarians for two weeks before vaccination to make sure of their health and for two years during the study in the Small Animal Teaching Hospital of Faculty of Veterinary Medicine at the University of Tehran, Tehran, Iran. Dog management, feeding, daily cleaning, nursing, and other conditions were similar in both groups performed at a high standard.

Rabdomun, a commercial BHK-21 cell-cultured vaccine made by Schering-Plough Animal Health, Madrid, Spain, was injected intramuscularly (tight muscle) in the first group (Nos. 1-6). Another cell culture vaccine, BHK-21C13, was derived from the PV-CEPANZO strain of fixed rabies passaged virus in young rabbits (PV-PARIS, passage 11 then passaged in BHK-21). This vaccine, made by the Pasteur Institute of Iran, was appropriately injected into a similar muscle in the second group of dogs (Nos. 7-12).

Rapid fluorescent focus inhibition test

The immune response of each dog was tested monthly for 18 months after vaccination by their serum samples. The blood serum were immediately separated and carried to the WHO Collaboration Center for Reference and Research on Rabies, located in the Pasteur Institute of Iran, Tehran, Iran. Serum-neutralizing antibody titers produced against rabies virus were determined by rapid fluorescent focus inhibition test (RFFIT). This method measures only that antibody that will kill live rabies virus. Patient serum is diluted out, and each dilution is mixed with live rabies virus. The mixtures are then put onto a cell culture to detect what virus was not killed by the serum. According to a rabies authority, the RFFIT test is the only internationally approved procedure for measuring rabies neutralizing antibody.

Statistical analysis

Repeated measure of analysis of variance (ANOVA) was used for comparison of variability of titers in two groups over 18 months by SPSS program (version 16). The confidence level was 0.95.

Results

On the basis of statistical analysis, the results of this study indicated that the maximum of mean rabies neutralizing antibody titers were observed exactly in the 6th month post vaccination in both groups, 9.2 IU/ml in group one vaccinated with

Spain Rabdomun compared to 12.60 IU/ml in group two vaccinated with the BHK-cell culture vaccine manufactured by the Pasteur Institute of Iran (Fig. 1). Neutralizing rabies antibody titer equal to and/or higher than 0.5 IU/ml was considered protective based on WHO protocol. The titer efficacy was 100% in both groups, up to 17 months after vaccination. However, in the 18th month post vaccination, the mean antibody titers were 0.6 in the first group that received the Spanish vaccine and 0.65 in the second group that received the Iranian vaccine. According to the results, at the end of the study (18th months) serum titer in only one dog (No. 7) was less than protective level. Two animals in group one (No. 2 and No. 5) also had serum titers less than the protective level. The mean of post vaccination antibody titer was not different between the two groups (P=0.35).

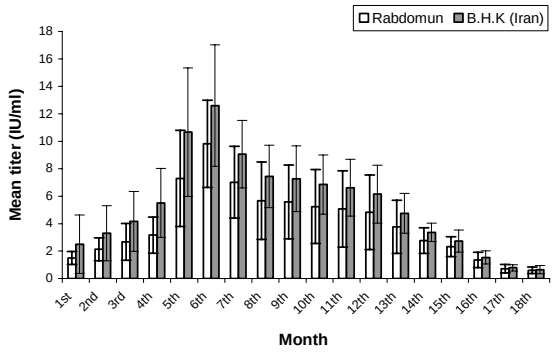

Fig. 1: Neutralizing antibody titer mean (IU/ml) after vaccination with Iranian and Spanish vaccines in two experimental dog groups over 18 months as a field trial experimental condition

Discussion

According to WHO report, each year 15 million people undergo anti rabies therapy following dog biting and almost 55000 people die because of delays in therapies or misdiagnosis (WHO, 2009). Carnivores are the main reservoir of the disease in Iran. In the north parts of Iran beside the shore of the Caspian Sea, foxes and gaggles have the main roles in transmission of the disease, while wolf are responsible for it in the west part. Rabies transmission in Iran is basically done by dogs, so 90% of the biting reports are on stray dogs (Rashidi, 2004). In 2004 about 88965 people (87.7%) out of 113542 animal bites are reported to have been bitten by dogs (Simani, 2004). Regarding some surveys held in the rabies research department of Iran Pasteur Institute, treatment of rabies has increased from 29860 cases in 1990 to 93216 cases in 2001. Other reports show that, in Iran the treatment has risen from 57070 cases in 1996 to 65632 in 1998. The majority of rabies cases have been reported from 1995 to 1997 in Chahar Mahal-o-Bakhtyari province in Iran (Simani, 1998). The present reports confirm that regular rabies vaccination is an important factor in pet animals for prevention programs. Using cell-cultured vaccines not only leads to short term therapy, but also causes an efficient immunity, whereas, it has no contraindication or side effects (Monaco *et al*., 2006).

The present study was performed on 12 dogs under controlled condition to compare cell-cultured vaccines made in Iran with the foreign one. The results of this study indicated that both vaccines cultured on BHK cells are safe and potent with appreciative immunity duration in the experimental dogs at least 15 months after the first vaccination. However, due to the vast diversity of ecological, geographical, and epidemiological aspects in Iran we suggest a booster injection, preferably the 12th month after the first vaccination (instead of 15th months post vaccination). These data demonstrated that the efficiency and persistence of the Iranian vaccine, produced by the Pasteur Institute of Iran, were acceptable and it was comparable to other imported vaccines and can be used in different locations of Iran. The present study also suggests that the best immunity in all dogs can be obtained in both vaccines by annual injections. The minimum level of antibody titer was 0.80 IU/ml in dog No. 5 in group one (received BHK-21 Rabdomun) 16 months post vaccination, while it was 0.60 IU/ml in dog No. 7 in group two (received BHK-21 made by Pasteur Institute of Iran). Whether time of vaccination could be delayed to more than one year (16 months or more) needs more experiments. In a similar study in Tunisia on 15 dogs separated in 3 groups of 5, the efficacy of a French vaccine

(made in the Merial Institute) was compared with the Tunisia Pasteur Institute one. In the result, the Tunisian one made the antibody titer peak one month after injection, bringing the titer to below the protective level after 162 days (Bahloul *et al*., 2005). In the present study, the dogs had 10.66 UI/ml titer at the same time by the Iran Pasteur made vaccines. The difference can be due to the present adjutants (Aluminum hydroxide). In conclusion, antibody titer was started sooner and ended faster by the Tunisian vaccine. Another advantage of using the vaccine in this study was the lack of any hypersensitivity reaction after vaccination. This finding indicates that these two vaccines are safe, potent and efficiently acceptable for annual booster vaccinations against rabies in dogs. The Iranian Pasteur Institute vaccine can be a suitable alternative for the imported ones. Nowadays, recombinant vaccines have been supported more and can be considered as a suitable alternative of previous types of vaccines. Following the insertion of the coding sequence of GnRH into different locations within the rabies virus glycoprotein, the GnRH-carrying rabies viruses have significant potential in rabies and animal population control (Wu *et al*., 2009).

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