CLINICAL AND VACCINE IMMUNOLOGY, Sept. 2011, p. 1477–1479 Vol. 18, No. 9
1556-6811/11/$12.00 doi:10.1128/CVI.05090-11

Persistence of Rabies Antibody 5 Years after Postexposure Prophylaxis with Vero Cell Antirabies Vaccine and Antibody Response to a Single Booster Dose▽

Xiaowei Zhang,[1]† Zhenggang Zhu,[2]† and Chuanlin Wang[3]*

Urology Department, Peking University People's Hospital, Beijing, China[1]; Wuhan Centers for Disease Prevention and Control, Wuhan, China[2]; and Emergency Department, Peking University People's Hospital, Beijing, China[3]

Received 17 April 2011/Returned for modification 6 June 2011/Accepted 7 July 2011

This study was done to investigate the antibody response to a Vero cell antirabies vaccine, the persistence of antibody for 5 years, and the effect of a booster dose after this interval. From August 2005 to February 2011, a total of 195 patients were enrolled into our study due to an animal bite. The Essen intramuscular (i.m.) regimen, which is recommended by the WHO for modern vaccines used in postexposure treatment, was adopted in this study. Blood samples were obtained on day 0, day 7, day 14, day 45, year 1, year 2, year 3, year 4, year 5, and year 5 plus 14 days. Immunogenicity was evaluated by the titration of neutralizing antibodies with a rapid fluorescent focus inhibition test (RFFIT). Seroconversion was expressed as the seroconversion rate (SCR). A secondary quantitative evaluation criterion, other than the seroconversion level, was the geometric mean titer (GMT). Of the 195 enrolled patients, 168 (86.4%) of them completed the whole study. No serious adverse reactions to the vaccine were reported during vaccination, the 5-year follow-up period, or revaccination. On day 14, the rabies antibody GMT value was 8.87 IU/ml in the vaccinees. During the next 5 years, the SCR in the ChengDa vaccine group gradually decreased to 34.0% at year 5, down from 90.5% at year 1. There was a significant booster effect: the GMT was 15.22 IU/ml on year 5 plus 14 days. Our findings demonstrate that the ChengDa rabies vaccine offers an alternative with a high degree of efficacy and yet limited side effects and ensures that the exposed patient will be on the safe side of the risk of rabies by the 14th day. Moreover, when followed by a booster dose 5 years later, it could boost the immunity. A further booster is effective in inducing a good neutralizing antibody response even after an interval of 5 years.

Although effective vaccines for the postexposure treatment of rabies are available (4), there are still about 50,000 to 60,000 human deaths annually. Rabies is a major public health problem in most of the developing world (3, 6). China, the largest developing country in the world, has endeavored tremendously to prevent rabies and manufacture vaccines. In 1981, the Semple vaccine was completely replaced by a locally produced tissue cell vaccine (TCV) in China (15). From 1990 to 1996, numbers of cases of human rabies were extremely low due to the nationwide rabies vaccination program (11), although the numbers of rabies cases increased considerably in recent years, due largely to more relaxed dog control measures. Approximately 5,000,000 persons undergo postexposure rabies vaccination annually (11). China accounts for almost two-thirds of the total postexposure prophylaxis (PEP) used in Asia, and the locally produced tissue culture vaccine is safe and relatively inexpensive (7).

Currently, the most prevalently used rabies vaccine in China is the purified Vero cell rabies vaccine (PVRV; Liaoning ChengDa Biological Co., Ltd., Shengyang, China). The Vero cell line has a long and successful history of use for the production of rabies and polio vaccines worldwide (8). The ChengDa rabies vaccine is grown on a Vero cell line utilizing the L. Pasteur 2061 strain of rabies virus. It is inactivated with β-propiolactone (BPL), lyophilized, and reconstituted in 0.5 ml of physiological saline. It is manufactured under good manufacturing practices (GMP) and strictly fulfills the WHO recommendations for potency. The ChengDa vaccine was licensed by the Health Ministry of China and the State Food and Drug Administration of China (SFDA) in 2002 and has been marketed throughout the country since that time.

Although a number of studies have been conducted to investigate the PVRV, there are few reports on the antibody levels, persistence, and booster responses in China (14), especially for the rabies vaccines manufactured in China. This report describes the antibody response to the PVRV, the persistence of antibody for 5 years, and the effect of a booster dose after this interval.

MATERIALS AND METHODS

The study (clinical trial registry no. NCT01173302) was conducted in the Emergency Department, Beijing University People's Hospital, and clinics for rabies prevention, Wuhan Centers for Disease Prevention and Control, Wuhan, China. The antibody evaluation was performed primarily at the Chinese National Institute for the Control of Pharmaceutical and Biological Products. The local ethics committee approved this study.

From August 2005 to February 2011, a total of 195 patients were enrolled into our study due to an animal bite. All of them were subjected to antirabies vaccination without rabies immunoglobulin. None of the included subjects had a detectable vaccination antibody titer prior to inclusion. The exclusion criteria were as follows: patients with primary or acquired immunodeficiency, patients taking corticosteroids, rabid patients, and patients who were simultaneously enrolled in other clinical studies. All patients gave their informed consent to

* Corresponding author. Mailing address: Emergency Department, Peking University People's Hospital, No. 11 Xizhimen South Street, Beijing, China. Phone: 86-13911883012. Fax: 86-01068318386. E-mail: erwangchuanlin@gmail.com.

† X.Z. and Z.Z. contributed equally to the study.

▽ Published ahead of print on 13 July 2011.

TABLE 1. GMT and SCR characteristics

Time point after primary immunization	No. of patients	Range of titers (IU/ml)	GMT (IU/ml)	Seroconversion rate (%)
Day 0	195	<0.5		0
Day 7	195	0.2–14.5	0.56	41.2
Day 14	195	1.7–23.7	8.87	100
Day 45	195	2.9–35.0	16.13	100
Yr 1	183	0.3–19.5	1.79	90.5
Yr 2	177	0.2–17.2	1.44	60.5
Yr 3	174	0.1–13.1	1.21	49.1
Yr 4	171	0.0–11.0	0.99	41.5
Yr 5	168	0.0–10.2	0.81	34.0
Yr 5 + day 14	168	1.3–45.2	15.22	100

participate in the study. In the current study, the ChengDa rabies vaccine was used. This vaccine is prepared with Vero cells with a registered potency of 4.5 IU/0.5 ml. The Essen intramuscular (i.m.) regimen, which is recommended by the WHO for modern vaccines used in postexposure treatment, was adopted in this study. This regimen comprises one dose of rabies vaccine administered on each of days 0, 3, 7, 14, and 28 by i.m. injection into the deltoid region. Furthermore, in this study, subjects were revaccinated at year 5. Blood samples were obtained on day 0, day 7, day 14, day 45, year 1, year 2, year 3, year 4, year 5, and year 5 plus 14 days. The blood sera were coded and sent for testing. Immunogenicity was evaluated by the titration of neutralizing antibodies with a rapid fluorescent focus inhibition test (RFFIT) (16) in a quality-controlled laboratory (National Institute for the Control of Pharmaceutical and Biological Products). The primary qualitative evaluation criterion was seroconversion, defined by a WHO-recommended antibody titer of 0.5 IU/ml. Seroconversion was expressed as the seroconversion rate (SCR). A secondary quantitative evaluation criterion, other than the seroconversion level, was the geometric mean titer (GMT).

SPSS 11.5 was used for statistical analysis, and a P value of <0.05 was considered significant. The analysis of the evolution of the SCR from day 0 to year 5 plus 14 days used the actuarial method, with an event being a titer that falls below 0.5 IU/ml. The calculation of GMT values began with the logarithmic transformation of the antibody titers.

RESULTS

Of the 195 enrolled patients, 168 (86.4%) of them completed the study, including vaccination, 5-year follow-up, revaccination, and consecutive antibody tests. The number of subjects over the 5-year period is detailed in Table 1. The number of males and females were similar (male-to-female ratio of 88 to 80), and the average age of the patients was 39.3 years (standard deviation [SD], ±7.4 years). The main reason for discontinuation was unwillingness for further on-site follow-up. Based on the telephone interview, these dropout subjects were free from rabies and vaccine-related adverse events.

Although safety was not systematically sought, no serious adverse reactions to the vaccine were reported during vaccination, the 5-year follow-up, and revaccination. The vaccine appeared to be safe and was well tolerated by the patients. No immediate reaction to immunization (within 30 min of injection) was reported after any of the doses.

In this study, from day 14, all patients had levels of neutralizing antibody to rabies virus in excess of adequate titers (>0.5 IU/ml), as defined by the WHO. On day 14, the rabies antibody GMT value was 8.87 IU/ml in the ChengDa vaccine group. The evolutions of the GMT values are shown in Table 1, along with the seroconversion rates at each time point. After the third vaccine dose on day 7 (see the day 14 GMT value) and after the fourth and fifth vaccine doses (see the day 45 GMT value), there were considerable increases in antibody titers. During the next 5 years, the SCR in the ChengDa vaccine group gradually decreased to 34.0% at year 5, down from 90.5% at year 1. However, after the year 5 booster, the SCR increased to 100%. There was a significant booster effect: the GMT was 15.22 IU/ml on year 5 plus 14 days. In the meantime, the 66% seronegative subjects at year 5 received a booster injection and had their titers measured 14 days thereafter: all of them seroconverted again.

DISCUSSION

In this study, we confirmed with a series of patients with PEP that the immunogenicity, antibody persistence, and booster response of the ChengDa rabies vaccine are satisfactory. As an observational study, the naturalistic design of the study enabled us to recruit patients seeking treatment for rabies exposure under conditions as close as possible to those of actual clinical practice. The demographic characteristics of the study population correspond to those expected under field conditions (12, 13). Follow-up of the patients in this study was relatively good: although nearly 15% of the patients were lost for the antibody test at year 5 plus 14 days, a total of 168 patients still participated in the final follow-up, which already offers adequate data for an overall analysis.

Most of the previous studies on the persistence of immunity after PEP and the effect of a booster dose have been carried out after intervals of 2 to 3 years (9, 10). Some studies may have had longer observational periods: Briggs and Schwenke (2) studied the persistence of rabies vaccine immunogenicity over a 9-year period, and Bahmanyar et al. conducted a study to demonstrate the effectiveness of a single booster dose 4 years after postexposure prophylaxis with a human diploid cell vaccine (HDCV) (1, 5). However, no solid evidence concerning the immunogenicity and booster response of rabies vaccines manufactured in China has been found. The present study is the first to demonstrate the persistence of immunogenicity for up to 5 years after postexposure rabies vaccination with the ChengDa vaccine.

The most significant findings of our study are that (i) in the absence of any further booster dose, 34.0% of the vaccinees still had antibody titers higher than 0.5 IU/ml at year 5; (ii) antibody levels rose markedly after the year 5 booster, and the GMT reached 15.22 IU/ml; and (iii) the antibody level on day 14 was more than double that achieved after the previous booster dose 5 years earlier.

Our findings demonstrate that the ChengDa rabies vaccine offers an alternative with a high degree of efficacy and yet limited side effects and ensures that the exposed patient will be on the safe side of the risk of rabies by the 14th day. Meanwhile, when followed by a booster dose 5 years later, it could give adequate immunity. A further booster is effective in inducing a good neutralizing antibody response even after an interval of 5 years.

ACKNOWLEDGMENTS

This study was supported by research and development funds from Beijing University People's Hospital. This study was funded by Peking University People's Hospital research and development funds (RDB 2009-26).

We especially thank the colleagues from the Wuhan Centers for Disease Prevention and Control, Wuhan, China, for their support.

We hereby state that we do not have any conflicts of interest with other people or organizations that could inappropriately influence this work.

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