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Ineffectiveness of rabies vaccination alone for post-exposure protection against rabies infection in animal models

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ABSTRACT

Most reported vaccination failures among rabies-exposed patients were due to fail to timely co-administer rabies immunoglobulin (RIG). Considering that such protection failure might be caused by low antigen titers in the vaccine, scientists improved antigen titers to 4.0 IU or even higher, yet the failure remained. Therefore, it becomes vital to develop more efficacious vaccine against rabies. In our evaluation of a novel PIKA rabies vaccine, we used multiple animal models (beagles, golden hamsters and Kunming mice) to mimic post-exposure scenarios. All animals were challenged with wild-type rabies virus, followed by vaccination with either rabies vaccines commercially available or PIKA rabies vaccines. As 100% of animals survived after administration of traditional rabies vaccines and rabies immunoglobulin, 80% of animals survived with rabies immunoglobulin alone. Strikingly, animals receiving traditional rabies vaccines alone showed extremely low survival rates, indicating insignificant benefit for exposed animals ($p > 0.05$, compared to unvaccinated control groups). To the contrary, 40–80% of animals receiving the experimental PIKA rabies vaccines were protected ($p < 0.05$, compared to unvaccinated control groups). If the above results are fully confirmed, we may conclude that currently as high as 99% of post-exposure patients who are seeking protection against rabies, but only receiving rabies vaccination, could be meaningless.

1. Introduction

Rabies is a zoonosis caused by rabies virus, a non-segmented, negative-strand lyssavirus within the Rhabdoviridae family (Barkhouse et al., 2015). It is transmitted from infected animal to humans through a bite or scratch contaminated with saliva-borne virus, or through intact mucous membrane (Manning et al., 2008). The mortality rate is almost 100% after the onset of clinical symptoms.

On Dec 10, 2015, World Health Organization (WHO) and the World Organization for Animal Health (OIE), in collaboration with the UN Food and Agriculture Organization (FAO) and the Global Alliance for the Control of Rabies (GARC), launched a global framework to eliminate rabies by 2030. The framework emphasized that massive vaccination of dogs in combination with management of stray dogs was the most cost-effective strategy to eliminate the disease. It has been confirmed that immunization in 70% of dogs can reduce human cases to zero in developed countries (Coleman and Dye, 1996; The, 2015). However, it is very difficult to ensure the vaccination rate of dogs in most developing countries, considering the ecological characteristics of dogs, lack of governmental coordination and huge overhead.

Current strategies to prevent human rabies infection recommended by WHO include wound cleaning, post-exposure vaccination and inoculation of rabies immunoglobulin (RIG) around the wound site for severe injury (World Health, 2013). Yet nearly 55,000 people still die from the disease annually and over 150 countries are affected (Yin et al., 2013). RIG administration in the post-exposure regimen is regarded as mandatory for “Category III” exposures (bites or scratches that break the skin and contamination of mucosae with saliva). However, the supply shortage and the high cost in many countries have led to low clinical use of RIG during post-exposure therapy. Uwanyiligira et al. evaluated the adequacy of rabies post-exposure therapy received by patients who consulted the Travel Clinic of the University Hospital, Lausanne, Switzerland (Uwanyiligira et al., 2012). They found that only 7 received human

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rabies immunoglobulin (HRIG) in the cohort of 50 patients of whom HRIG was indicated. Li et al. investigated the use of rabies vaccine and HRIG in Fujian, China during 2008–2013 (Li, 2014). They found that the injection coverage rate of HRIG was only 24.5% for "Category III" exposure. Another study conducted during 2007–2012 in Chongqing, China, found that the injection coverage rate of HRIG was only 2.6% (Su et al., 2015). On the other hand, Bahloul et al. reported that vaccination alone was insufficient for post-exposure protection against rabies infection where traditional vaccine protected 40% of mice (Bahloul et al., 2003). Lin et al. found that rabies vaccine with or without alum adjuvant failed to protect mice infected with rabies (Lin et al., 1993). Xu et al. compared the protective effects of two traditional vaccines on Kunming mice post virus challenge where they found that the protection rate was 0% (Wang, 2010). A study involving 725 human rabies cases in Guangxi province of China reported 197 deaths due to vaccination failure; amongst which 78.27% cases died prior to the 4th injection administered (Wang, 2010), indicating that vaccination alone couldn't effectively protect humans from rabies virus infection.

Recently we reported a novel PIKA rabies vaccine, which could induce higher rabies virus neutralizing antibody (RVNA) titer, stronger cellular immunity and better protection than the traditional rabies vaccines (Zhang et al., 2016). We took a systemic approach by conducting a series of animal experiments initially aiming at better characterizing the new class vaccine, however, the dramatically different protection efficiency conferred by the use of traditional rabies vaccine alone versus co-use of traditional rabies vaccine and rabies immunoglobulin illustrated that traditional rabies vaccine alone could not provide protection under post-exposure settings.

2. Materials and methods

2.1. Animals

Kunming mice and Golden hamsters were purchased from Changchun Institute of Biological Products Co., Ltd. Beagles were purchased from Beijing Rixin Technology Co., Ltd. The neutralizing antibodies in serum of Beagles were tested using FAVN test (fluorescent antibody virus neutralization test) as described previously (Cliquet et al., 1998) and showed negative prior to the tests. All animals were treated according to the regulations of Chinese law and local Ethical Committee.

2.2. Viruses and cell lines

Wild virus strain BD06 in viral challenge test was isolated from an infected dog and maintained by Veterinary Institute, Academy of Military Medical Sciences, China. Vero cell line was purchased from National Institutes for Food and Drug Control (China).

2.3. Vaccines

Purified Vero Cell Rabies Vaccines were manufactured by Liaoning Cheng Da Co., Ltd (PVRV-CD) and Liaoning Yisheng Biopharma Co., Ltd (PVRV-YS). Purified chick embryo cell rabies vaccines were manufactured by Novartis (PCECV). PIKA rabies vaccine (PIKA-RV) was obtained by propagating the rabies virus in Vero cell line before mixing with PIKA adjuvant and manufactured by Liaoning Yisheng Biopharma Co., Ltd. HRIG was purchased from Wuhan Institute of Biological Products Co., Ltd.

2.4. Post-exposure efficacy test in Kunming mice

Kunming mice were randomized into 3 groups (n = 30) with different immunization schedules. Mice were first i.m. infected with 50 LD_{50} wild rabies viruses (BD06 strain) at the right hind legs, then treated with saline or PVRV-CD (Lot Number: 201407233), or PIKA-RV (Lot Number: II-20130901) respectively. For saline and PVRV-CD groups, animals were injected with 0.1 ml of solution at the left hind legs on days 0, 3, 7, 14 and 28 post exposure. For PIKA-RV group, animals received single-dose injection (0.1 ml) at each hind leg on day 0 and 2 post exposure and single-dose injection at the left hind leg on day 7 post exposure. All mice were observed for 30 days post exposure to monitor the development of rabies-specific symptoms and mortality. The succumbed animals were confirmed by direct fluorescent antibody test (DFA) test.

2.5. The first post-exposure efficacy test in golden hamsters

Female golden hamsters were randomized into 4 groups (n = 10) with different immunization schedules. Animals were first i.m. infected with 50 LD_{50} wild rabies viruses (BD06 strain) at the right hind legs, then treated with saline, PCECV (Lot Number: 1983), PVRV-YS (Lot Number: 201206070-1), or PIKA-RV (Lot Number: V-26) respectively. For saline, PCECV, and PVRV-YS groups, animals were injected i.m. with a volume of 0.1 ml at the left hind legs on days 0, 3, 7, 14 and 28 post exposure. For PIKA-RV group, animals received two separate i.m. injections on day 0 and 2, e.g., single-dose injection (0.1 ml) at each hind leg, and one single-dose injection (0.1 ml) i.m. at the left hind leg on day 7 post exposure. All animals were observed for 45 days post exposure for the development of rabies-specific symptoms and the survival rate was calculated. The succumbed animals were confirmed by DFA test.

2.6. The second post-exposure efficacy test in golden hamsters

This experiment was repeated with 15 animals in each group. Animals were first i.m. infected with 50 LD_{50} wild rabies viruses (BD06 strain) at the right hind legs, then treated with saline, or PCECV (Lot Number: 1995), or HRIG (Lot Number: 20120505) or PIKA-RV (Lot Number: II-20130401) respectively. For HRIG group, animals were administered i.m. with 1.5 IU of HRIG at each site of right and left hind leg 2 h post exposure. The other groups were treated similarly with the first post-exposure efficacy test in golden hamsters.

2.7. The first post-exposure efficacy test in beagles

Four groups of Beagles, 15 Beagles in each group were infected i.m. with 120,000 LD_{50} of BD06 rabies viruses at the masseter muscle, then immunized with different strategies post infection. Animals were administered with 1 ml of PBS, PCECV (Lot Number: 1897) or PIKA-RV (Lot Number: 20110201) at the masseter muscle on days 0, 3, 7, 14 and 28 post exposure. In addition to the above procedures, treatment for PCECV + HRIG group included a one-time injection of HRIG (200IU) two hours post exposure. Serum samples were collected on days 0, 7, 14 and 28 and the RVNA titers were evaluated using FAVN test as described previously (Cliquet et al., 1998), which is a standardized test recommended by WHO. Infected and treated animals were observed for 45 days post exposure for the development of rabies-specific symptoms or death and the survival rate was calculated. The succumbed animals were confirmed by DFA test.

2.8. The second post-exposure efficacy test in beagles

This test was performed with 10 animals in each group i.m. challenged with 160,000 LD_{50} of virus, a higher dose than 120,000 LD_{50} used in the first test. After challenge, animals were