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Original research

Study on immune persistence of the CTN-1V strain rabies vaccine in humans

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ABSTRACT

This study is a single-arm, single-center phase IV clinical trial on a rabies vaccine that has been marketed in China. The Vero cells and CTN-1V strain are used in the rabies vaccine product. The purpose of this study was to investigate the safety, immunogenicity and immune persistence of this product. One hundred and forty-nine participants were enrolled to the study, all of whom were included in the safety analysis set (SS), among which 116 participants were included in the protocol analysis set (PPS), One hundred and fifteen participants were included in the 6-month immune persistence analysis set (IPS6) and 111 in the 12-month immune persistence analysis set IPS12. Results showed that: 1) In the SS analysis set, adverse reactions were mainly pyrexia and pain at the vaccination site, the severity of which were mostly grade 1, and concentrated in 0–3 days after vaccination. No grade 3 or above adverse events and serious adverse events (SAE) related to the experimental vaccine were observed. 2) In the PPS analysis set, the antibody positive conversion rate reached 100% at 14 days after full immunization of the pre-immunized negative population; The antibody geometric mean titer (GMT) (95% CI) was 14.82 (13.00, 16.90). 3) The positive rate of serum neutralizing antibody was 93.91 % and the GMT at 1.58 IU/ml at 6 months after full immunization. The positive rate of neutralizing antibody was 85.59 % and GMT at 1.30 IU/ml at 12 months after immunization. Our results show that the human rabies vaccine with the CTN-1V strain and Vero cells as matrix had good safety, immunogenicity and immune persistence in our study.

1. Introduction

Rabies is a viral zoonotic disease responsible for an estimated 59 000 human deaths and over 3.7 million disability-adjusted life years (DALYs) lost every year.[1] Once clinical symptoms are present, the prognosis for survival is poor and death is almost inevitable. One of the most important elements in the effective control of human rabies is the use of vaccine and human rabies immunoglobulin (HRIG).

The rabies vaccine was first used in the 1880s, after the nerve tissue vaccine, avian embryo vaccine and cell culture-based vaccine were used successively. The WHO Position Paper on rabies vaccine has been updated progressively. It does not recommend the use of nerve tissue vaccines for pre-exposure prophylaxis (PrEP) due to its severe adverse reactions in 2002. First recommended were purified chick embryo cell vaccine and purified Vero cell rabies vaccine being less expensive have comparable characteristics with the human diploid cell rabies vaccine which was introduced in 1967.[2,3] By 2007, the Position Paper recommended the use of cellculture-based vaccines (CCVs) instead of nerve tissue-based vaccines for both PrEP and post exposure prophylaxis (PEP). CCVs consist of virus that has been inactivated following propagation in cell cultures or in embryonated eggs, such as human diploid fibroblasts, fetal rhesus cells, primary Syrian hamster kidney cells, Vero cells (African green-monkey kidney cells), chick embryo cells or embryonated duck eggs.[4] In the latest version of the WHO Position Paper

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in 2018, the use of modern, concentrated, purified cell culture and embryonated egg-based rabies vaccines (CCEEVs) are clearly recommended. The CCEEVs contain inactivated rabies virus (RABV) that has been grown in embryonated eggs (e.g. embryonated duck or chicken eggs) or in cell cultures (e.g. primary chick embryo cells, Vero cells or human diploid cells).[5]

The use of human diploid cell-based rabies vaccine represents a dividing line between nerve tissue-based and cell culture-based vaccines. With the further development of science and technology in the 21st century, primary chick embryo cell and Vero cell rabies vaccine are widely used. In this study, we have used Vero cells as cell substrate for our rabies vaccine product.

Viral strains used for the production of the rabies vaccines incude, but are not restricted to, the Pitman Moore virus, Pasteur Virus, the Vnukovo −32, the Flury LEP, and the CTN.[6]

The rabies virus CTN strain was isolated and stored by The National Institute of Food and Drug Control (NIFDC) of China. The virus was isolated from the brain tissues of patients who had died of rabies in 1956. After 56 successive generations in mouse brain, it became a fixed virus, named CTN-M strain. Subsequently, it was adapted in KMB-17 cells for 50 generations and became the CTN-1 strain. Later, it was adapted in Vero cells. The virus titer reached more than 8.0 $lgLD_{50}$/mL, with high yield and good immunogenicity, and named the CTN-1V strain [7].

This research is about the freeze-dried rabies vaccine for human use that has been marketed in China. It is a vaccine product produced with the CTN-1V strain and Vero cells as matrix. The safety, immunogenicity and immune persistence of the vaccine after five intramuscular injections were investigated in this study.

2. Methods

2.1. Study design and participants

This was a single arm, single center phase IV clinical trial in China (Clinical trials. gov, NCT05547815). The implementation period of this clinical trial was from December 2019 to November 2021, which conforms to the requirements of the Helsinki Declaration and Good Clinical Practice (GCP).

149 Volunteers aged 10 to 60 were recruited for the experiment. Exclusion criteria include: vaccinated with rabies vaccine or rabies immunoglobulin; Immunodeficiency disease; No spleen; Pregnancy or planned pregnancy; Allergy to any component of the test vaccine; Serious congenital malformations or chronic diseases that may interfere with the study; Suffering from systemic diseases; People who are participating in or planning to participate in other clinical trials.

2.2. Vaccines

The vaccine is a lyophilized, sterile, purified inactivated rabies vaccine prepared on Vero cells. Each reconstituted dose of 0.5 ml contained Inactivated CTN-1V strain Rabies virus, with a potency of $\geq$2.5 IU (NIH potency test). Batch numbers 201902002, expiry 13 February 2021, the vaccine potency is 4.4IU/dose.

Persons should be injected with 1 dose of this vaccine on Day 0, Day 3, Day 7, Day 14 and Day 28, and a total of 5 doses should be administered throughout the immunization procedure.

2.3. Immunogenicity assessment

All 149 subjects were immunized with 5 doses of vaccine in the whole course. On the 14th day, the 6th month and the 12th month after the last dose of immunization, blood samples were collected to detect anti rabies virus neutralizing antibodies (RVNA). The detection method was rapid fluorescent focus inhibition test (RFFIT).[8,9] Which was detected by China National Institutes for Food and Drug Control (CNIFDC).

The primary end point was to evaluate the antibody seroconversion rate on the 14th day after the last dose of immunization, and the antibody seroconversion rate levels at the 6th and 12th months; The secondary end point was the geometric mean titer (GMT) of anti-rabies virus neutralizing antibody on the 14th day after the last immunization, and the GMT of antibody in the 6th and 12th months.s.

2.4. Safety

All adverse events within 30 days from the first dose of vaccine to the full course of vaccination, including solicitation events within 30 min after each dose of vaccine and 7 days after vaccination (if the interval between the current vaccination and the next vaccination is less than 7 days, the solicitation period shall be subject to the actual interval between two levels of vaccination), all non-solicitation events within 0–30 days and all serious adverse events within 6 months after the full course of vaccination.

Collected adverse events include local reactions such as pain, induration, erythema, swelling, pruritus, rash, physical activity disorder caused by them, and systemic events such as fatigue, headache, nausea, vomiting, vertigo, abdominal pain, myalgia, arthralgia, hypersensitivity, and pyrexia.

Non solicitation adverse events include any adverse events except solicitation events (or solicitation period), such as acute diseases, accidental injuries, etc.

30 min of on-site observation after each vaccination, the researcher recorded the observation results, and the subjects recorded all adverse events 0–3 days, 0–7 days and 8–30 days through diary cards and contact cards, and collected all serious adverse events from the first dose of vaccine to 6 months after the whole course of vaccination by means of the researcher's telephone interview and the subject's active report, including pregnancy events from the first dose of vaccine to 1 month after the whole course of vaccination.

3. Statistical methods

Measurement data were statistically described by means, median, standard deviation, maximum and minimum values. Counting or grading data are expressed in terms of frequency.

All statistical analysis was performed using statistical software SAS 9.4.

4. Results

149 subjects received at least one dose of the experimental vaccine and entered the total safety analysis set (SS) and the full analysis set (FAS). 116 subjects received blood samples 14 days after full vaccination and entered the per-protocol set (PPS). 115 subjects underwent blood collection 6 months after completing full immunization and entered the 6-month immune persistence set (IPS6). 111 subjects received blood collection 12 months after completing full immunization and entered the 12 month immune persistence set (IPS12).

4.1. Participants

All enrolled subjects, including 48 male subjects and 101 female subjects, were aged between 10 and 60 years old. For details, see Table 1. All subjects had normal pre-treatment body temperature, blood pressure, cardiopulmonary auscultation, and skin examination Immunogenicity.

Table 1
Baseline demographic characteristics of subjects in each analysis set.

Analysis item	analytic set		
	FAS (N = 149)	PPS (N = 116)	SS (N = 149)
Age (year)			
N (Missing)	149 (0)	116 (0)	149 (0)
Mean (SD)	35.87 (15.02)	35.39 (15.40)	35.87 (15.02)
Median	39.00	39.00	39.00
Min, Max	9.0 , 59.0	10.0 , 59.0	9.0 , 59.0
Gender			
male n(%)	48 (32.21)	36 (31.03)	48 (32.21)
female n(%)	101 (67.79)	80 (68.97)	101 (67.79)
total (Missing)	149 (0)	116 (0)	149 (0)
Height (cm)			
N (Missing)	149 (0)	116 (0)	149 (0)
Mean (SD)	154.0 (8.9)	154.2 (8.3)	154.0 (8.9)
Median	154.0	154.0	154.0
Min, Max	120 , 178	135 , 178	120 , 178
Weight (kg)			
N (Missing)	149 (0)	116 (0)	149 (0)
Mean (SD)	56.90 (13.26)	56.75 (13.13)	56.90 (13.26)
Median	58.00	57.00	58.00
Min, Max	25.0 , 88.0	25.0 , 88.0	25.0 , 88.0

4.2. Immunogenicity

4.2.1. Serum neutralizing antibody GMT on 14 days after full immunization

In PPS, serum neutralizing antibody GMT was 14.82 IU/ml, 15.25 IU/ml and 22.51 IU/ml in pre-immunization negative, whole and pre-immunization positive populations at 14 days after full immunization, respectively. The serum neutralizing antibody GMT of all the populations at 14 days after full immunization was shown in the Table 2. The seroconversion rate of serum neutralizing antibody was 100% after 14 days of full immunization.

4.2.2. Immune persistence

A total of 115 subjects completed the whole immunization after 6 months of immune persistence observation, 111 subjects completed the whole immunization after 12 months of immune persistence observation.

At 6 months and 12 months after full immunization, the serum neutralizing antibody seroconversion rates were 93.91% and 85.59% respectively, and the GMT values were 1.58 IU/ml and 1.30 IU/ml respectively. See Table 3 for details.

4.3. Safety

4.3.1. Adverse events (by incidence)

Among 149 subjects admitted to SS in this study, adverse reactions were mainly manifested as fever and pain at the vaccination site, with the severity of grade 1 and concentrated in 0–3 days. No grade 3 or above adverse events and SAE related to the experimental vaccine were observed.

Adverse events associated with experimental vaccines, as recommended by the Council for International Organizations of Medical Sciences (CIOMS), Very common (≥10%), Common (1.0%–10%, including 1.0%), Uncommon(0.1%–1.0%, including 0.1%), Rare (0.01%–0.1, including 0.01%), Very rare (<0.01%), the adverse reactions in this test are analyzed according to this classification below. For details, see Table 4.

In this study, the total incidence of AE ≥1.0% included: vaccination site: Pruritus, Swelling; Non-vaccination sites: Asthenia, Headache, Abdominal pain, Nausea, Vomiting, Arthralgia, Myalgia, Vertigo, Hypersensitivity.

Uncommon (0.1%–1.0%, including 0.1%): Vaccination site: Vaccination site erythema, Induration. Non vaccination site of the vaccine: vomiting.

Table 2
Serum neutralizing antibody GMT at 14 days after full immunization in PPS set.

analytic set	Analysis population	experimental group		
		number	GMT	95%CI
PPS	Whole population	116	15.25	13.45 , 17.31
	Pre-immunized negative population	108	14.82	13.00 , 16.90
	Pre-exemption positive population	8	22.51	14.22 , 35.65

5. Discussion

The pre-immunization serum-negative population in PPS, the seroconversion (≥0.5IU/ml) rate of serum neutralizing antibody was 100% and the GMT of serum neutralizing antibody was 14.82 IU/ml at 14 days after full immunization, the seroconversion rate of serum neutralizing antibody at 6 months after full immunization was 93.91%, and the GMT of antibody was 1.58 IU/ml. The seroconversion rate of neutralizing antibody was 85.59% and GMT was 1.30 IU/ml at 12 months after immunization.

The results of this study are comparable to those of other studies in recent years. A study conducted by Lei Zhang et al.,[10] using a 5-needle immunization program showed that the antibody positivity conversion rate was 100% at 42 days after the first dose of vaccination, and the antibody titer was greater than 10IU/ml. About 84.11% of the participants remained positive in half year. The positive rate decreased relatively to 58.62% in one year. Another study by Zhuhang Huang et al.,[11] in 2022, showed that the neutralizing antibody positive conversion rate after 12 months of immunization with a Vero cell-based rabies vaccine was 77.78%, and the neutralizing antibody evaluation level was 1.6IU/ml. Another study by Lei Zhou et al.[12] in 2022 was to investigate the immune persistence of three batches of Rabies vaccine produced with CTN-1V strain virus and Vero cell as the matrix. The results showed that the antibody positive conversion rate of three batches was 100% at 42 days after the first dose of immunization, and the antibody titers were 21.34IU/ml, 24.90IU/ml, 27.38IU/ml, respectively. The positive conversion rate of Neutralizing antibody in three batches after 12 months of immunization was 77.78, 84.75%, 91.67%, and the Neutralizing antibody titers were 1.34IU/ml, 1.44IU/ml, 1.48IU/ml, respectively. In a study conducted by Robert L. Jones and others in 2001,[13] the immune persistence study on CPRV showed that the antibody positive conversion rate was 100% at 42 days after the first douse of immunization, the titer was 16.9IU/ml, the positive conversion rate was 98.3%, the Neutralizing antibody titer was 3.4IU/ml in half a year, the positive conversion rate was 92.2%, and the Neutralizing antibody titer was 1.6IU/ml in one year.

The results obtained from this study are similar to the above data. The antibody positive conversion rate was 100% 14 days after the whole Rabies vaccine was immunized, and the antibody titer reached a high value, the average value was generally more than 10IU/ml. The antibody titer dropped significantly in 6 months, the average value of antibody titer was generally less than 4IU/ml, and the positive conversion rate was less than 100%. In 12 months, compared with 6 months, the antibody titer dropped slightly, but the positive conversion rate level would decline.

The safety observations of 149 people in this study showed that the highest proportion of adverse reactions were Pyrexia and pain, no grade 3 or above adverse events were observed. For the safety study of human rabies vaccine, a number of studies have shown that the highest incidence of local adverse reactions is pain, and most of the highest incidence of systemic adverse reactions is Pyrexia, in which the number of subjects in the study ranged from dozens to tens of thousands of people, the immunization methods included intramuscular injection (IM) and

Table 3
Antibody levels at 6 months and 12 months after full vaccination.

analytic set	antibody positive rate				antibody GMT		
	number	positive number	rate (%)	95%CI	number	GMT	95%CI
IPS6	115	108	93.91	87.86 , 97.52	115	1.58	1.29 , 1.93
IPS12	111	95	85.59	77.65 , 91.53	111	1.30	1.04 , 1.62

Table 4
Adverse events with incidence ≥1%.

frequency of occurrence	analysis item
≥10%	Pyrexia
	Vaccination site pain
1.0%–10%	Vaccination site pruritus
	Asthenia
	Vaccination site swelling
	Headache
	Abdominal pain
	Nausea
	Vomiting
	Arthralgia
	Myalgia
	Vertigo
	Hypersensitivity

intradermal injection (ID) , and the immunization procedures included four needle procedures and five needle procedures.[14–19] In addition, the results of a post-exposure immunization study showed that although the number of observers was small, only 90 people, the incidence of local Grade 3 adverse reactions reached 12%–43%.[20]

By comparing the results of this study with published research data, it is shown that this rabies vaccine product has good immune persistence and excellent safety. This study disclosed the immune persistence effect and the safety of a Rabies vaccine prepared with Vero cell as the cell matrix and CTN-1V strain as the virus strain, providing some data for reference to Rabies vaccine researchers around the world.

Ethical Approval

This study was approved by the Vaccine Clinical Trial Ethics Committee of Guizhou Provincial Center for Disease Control and Prevention China, approval number: GZ-20190001.

CRediT authorship contribution statement

Lidong Wang: Writing – original draft. **Jia Li:** Writing – original draft. **Qiuyue Mu:** Methodology. **Lei Zhu:** Data curation. **Yunpeng Wang:** Validation. **Ying Sheng:** Formal analysis. **Danhua Zhao:** Supervision. **Guoling Yang:** Formal analysis. **Xiaoqing Yu:** Investigation. **Xiaohong Wu:** Validation, Writing – review & editing. **Li Miao:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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