Study on the Quality of Lyophilized Human Diploid Cell Rabies Vaccine Using Microcarrier Technology

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ABSTRACT

The aim is to study the potency, purity, safety, and stability of the lyophilized human diploid cell rabies vaccine (HDCV). The viruses were harvested from infected human diploid cells (MRC-5 strain) which were cultured in a microcarrier bioreactor. After harvest, purification, inactivation and lyophilization, HDCV was produced. The potency of vaccines was measured by NIH method; the bovine serum protein residual content and the antibiotic residues were tested by ELISA method; the endotoxin content was detected by semi quantitative gel method; the safety of vaccine was determined in vivo. Among 6 batches of HDCV, the lowest immunizing potency was 4.79 IU/ml, whilst the highest was 6.03 IU/ml; the lowest bovine serum protein residual content was 12.41 ng/dose, whilst the highest was 31.74 ng/Dose; the content of antibiotic residues was from 2.20 ng/ml to 4.00 ng/ml; endotoxin levels were all lower than 50 EU/dose. All the mice and guinea pigs vaccinated were all alive, and the body weight of each mouse also increased. The stability was investigated by determining the water content and potency of the vaccine placed in 37±1 °C for 4 weeks and 2-8 °C for 48 months, respectively. The results indicate all the quality index accords with the standards of “Pharmacopoeia of the People's Republic of China 2010, 3 Volumes”. HDCV shows satisfactory potency, purity, safety, and stability.

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Abbreviations: HDCV, human diploid cell rabies vaccine; PM, Pitman-Moore; ELISA, enzyme linked immunosorbent assay; NIH, National Institutes of Health. STR, Short Tandem Repeat Analysis

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Introduction

Rabies is an acute zoonotic infectious disease caused by the rabies virus affecting the central nervous system. It is usually transmitted by a bite from an infected mammal \([1, 2]\). Rabies is virtually a 100% fatal disease whose incubation period is long \([3, 4]\). Misdiagnosis is quite frequently in the early phase, especially in children and subjects bitten by unknown animals \([5]\). Appropriate pre- or post- exposure rabies vaccination is the only effective method to control human rabies \([6]\). Yet most human rabies vaccines in the market have poor safety, as they are made from infinite cell lines which might have the risks of tumorigenicity \([7]\).

The potency, purity, safety, and stability of rabies vaccines are the key index of the quality. To investigate rabies antibody titer is a method of determining whether the rabies is preventable by rabies vaccines \([8]\). Thus, rabies antibody titer might be indicative of preventative and protective effects of rabies vaccines \([9]\). The ideal rabies vaccine can not only induce the cell immune responses, but also maintain enough long time of protection \([10]\).

Pitman-Moore (PM) virus strain is suggested to produce human diploid cell vaccine \([11]\). And MRC-5 has been already proved to be cultivated on Cytodex I microcarriers in a bioreactor \([12]\). The production process of the lyophilized human diploid cell rabies vaccine (HDCV) of Chengdu Kanghua Biological Products Co., Ltd. (Kangh) has been approved by the China Food and Drug Administration (FDA) in 2013. It is the first human rabies vaccine made from human diploid cells based on the bioreactor technology in China. Thus to evaluate the quality of HDCV is important. In this study, the quality index of 6 batches of HDCV was assessed.

Materials and methods

1. Material

PM virus strain and MRC-5 cell line were prepared from working cell bank of Kanghua Biological Products Co., Ltd. Cytodex I microcarriers were purchased from Pharmacia Ltd. Liquid seed batches and cells should be tested by STR.

2. Methods

2.1 Preparation of human diploid cell rabies vaccine

MRC-5 cells were thawed and cultured in a kolle flask, and then amplified in a microcarrier bioreactor. Then MRC-5 cells were infected by PM virus strain. The viruses were repeatedly harvested, concentrated, purified and inactivated. The rabies vaccines were produced after dilution, separation and lyophilization.

2.2 Determination of human diploid cell rabies vaccine

Test the potency, bovine serum protein residual content, antibiotic and endotoxin...
Figure 1: Potency results in different batches of HDCV

Result

1. Potency: Tested 6 batches of HDCV, and The potencies were 4.79-6.03 IU/ml. the results indicated a good immunogenicity (See Figure 1).

2. Purity: Tested the contents of bovine serum protein residues, antibiotic residues, and bacterial endotoxin of 6 batches of HDCV, and All the results were qualified for “Pharmacopoeia of the People's Republic of China 2010 English Edition, 3 Volumes” (See Table 1).
### Table 1: The results of the Purity of HDCV

<table>
<thead>
<tr>
<th>Batches No.</th>
<th>Bovine Serum Protein Residues (ng/Dose)</th>
<th>Antibiotic Residues (ng/ml)</th>
<th>Bacterial Endotoxin (EU/Dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20100101</td>
<td>12.41</td>
<td>4.00</td>
<td>&lt;50</td>
</tr>
<tr>
<td>20100102</td>
<td>31.74</td>
<td>3.30</td>
<td>&lt;50</td>
</tr>
<tr>
<td>20100203</td>
<td>14.35</td>
<td>2.70</td>
<td>&lt;50</td>
</tr>
<tr>
<td>20101007</td>
<td>27.46</td>
<td>2.70</td>
<td>&lt;50</td>
</tr>
<tr>
<td>20101008</td>
<td>25.73</td>
<td>2.20</td>
<td>&lt;50</td>
</tr>
<tr>
<td>20101009</td>
<td>13.57</td>
<td>3.30</td>
<td>&lt;50</td>
</tr>
</tbody>
</table>

3. **Safety test**: No abnormity was found. For the mice group, all the mice were healthy, and the body weight of each mouse increased; For the guinea pigs group, All the guinea pigs were healthy, and the body weight of each guinea pigs increased. It could conclude that the HDCV was safe.

4. **Stability**

4.1 Water contents in 37±1 °C at different timepoints of 0, 1, 2, 3, 4 weeks: Water contents of Lot 20100101 were 1.2%, 1.2%, 1.1%, 1.3%, 1.3%, respectively; and Water contents of Lot 20100102 were 1.6%, 1.5%, 1.6%, 1.7%, 1.8%, respectively; and Water contents of Lot 20100203 were 1.1%, 1.2%, 1.1%, 1.1%, 1.3%, respectively. Water contents of Lot 20100107 were 1.6%, 1.8%, 1.9%, 2.0%, 2.0%, respectively; and Water contents of Lot 20100108 were 1.5%, 1.6%, 1.7%, 1.8%, 1.7%, respectively. Water contents of Lot 20100109 were 1.7%, 1.8%, 2.0%, 2.2%, 2.1%, respectively.

4.2 Potencies in 37±1 °C at different timepoints of 0, 1, 2, 4 weeks: Potencies of Lot 20100101 were 5.3 IU/ml, 5.5 IU/ml, 4.7 IU/ml, 4.0 IU/ml, respectively; and potencies of Lot 20100102 were 5.0 IU/ml, 4.7 IU/ml, 5.3 IU/ml, 4.8 IU/ml, respectively; and potencies of Lot 20100203 were 6.0 IU/ml, 5.6 IU/ml, 6.2 IU/ml, 5.2 IU/ml, respectively. Potencies of Lot 20100107 were 5.2 IU/ml, 5.0 IU/ml, 4.6 IU/ml, 4.2 IU/ml, respectively; and potencies of Lot 20100108 were 6.0 IU/ml, 5.3 IU/ml, 5.5 IU/ml, 4.7 IU/ml, respectively; and potencies of Lot 20100109 were 4.8 IU/ml, 4.8 IU/ml, 4.2 IU/ml, 4.7 IU/ml, respectively (See Figure 2).

4.3 Potencies in 2-8 °C at different timepoints of 0, 12, 18, 24, 36, 42, 48 months: After 0, 12, 18, 24, 36, 42, 48 months, potencies of Lot 20100101 were 5.3 IU/ml, 5.5 IU/ml, 5.0 IU/ml, 4.7 IU/ml, 4.5 IU/ml, 4.0 IU/ml, 5.5 IU/ml, respectively; and potencies of Lot 20100102 were 5.3 IU/ml, 5.5 IU/ml, 5.0 IU/ml, 4.7 IU/ml, 4.5 IU/ml, 4.0 IU/ml, 5.5 IU/ml, respectively.
were 5.0IU/ml, 5.0IU/ml, 5.1IU/ml, 4.7IU/ml, 4.9IU/ml, 4.3IU/ml, 3.6IU/ml, respectively; and potencies of Lot 20100203 were 6.0IU/ml, 4.8IU/ml, 5.1IU/ml, 5.1IU/ml, 4.9IU/ml, 4.1IU/ml, 4.5IU/ml, respectively. After 0, 12, 18, 24, 36 months, potencies of Lot 20100107 were 5.2 IU/ml, 5.1IU/ml, 6.0IU/ml, 5.0IU/ml, 4.8IU/ml, respectively; and potencies of Lot 20100108 were 6.0IU/ml, 5.2 IU/ml, 5.7IU/ml, 5.2IU/ml, 5.6 IU/ml, respectively; and potencies Lot 20100109 were 4.8IU/ml, 4.7IU/ml, 4.5IU/ml, 4.4IU/ml, 4.7IU/ml, respectively. Potencies of batch number 20100107, 20100108, 20100109 after 42, 48 months were being assessed in progress (See Figure 3).

Figure 2: Potency results of HDCV in 37±1 °C at different timepoints

Figure 3: Potency results of HDCV in 2-8 °C at different timepoints

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Discussion

Presently, rabies occurs most commonly in China. Vaccination of rabies vaccine is the main and only therapeutic strategy to control the spread of the epidemic. In domestic market, the types of human rabies vaccines are mainly the Vero cell rabies vaccine and other animal cell culture vaccine \(^{[14, 15]}\). Nevertheless, if the exogenous DNA residues of the vaccines can not be strictly controlled, it might cause tumorigenesis. Few researches are focused on the quality of human diploid cell rabies vaccines made from healthy human embryo lung cells which have no risks of tumorigenesis \(^{[16]}\).

Traditional ways for human diploid cell rabies vaccines (HDCV) are produced in cell factories. However, the relatively independent culturing conditions in different cell factories might cause poor consistency of products quality. Furthermore, producing a batch of HDCV requires hundreds of manual operation steps for opening cell factories. The extensive manual processing might be a grand challenge to assure sterility. Instead, using microcarrier technology to produce a batch of HDCV in the bioreactor only needs two opening operation steps, consequently drastically reducing the risks of manual contamination.

To our best known, it is the first time to apply microcarrier technology into human rabies vaccine production process in MRC-5 cells infected by PM virus strain in China. PM virus strain from Wistar Institute and MRC-5 cell line from European Collection of Cell Cultures (ECACC) were introduced by Kangh in China. Based on the microcarrier technology, HDCV has been developed by Kangh since 2013.

Tests aimed at determining the quality of 6 batches of HDCV. The lowest potency was 4.79 IU/ml, while the highest was 6.03 IU/ml among all the 6 batches. The protein contents were all < 32.00 ng/Dose; the antibiotic residues contents were < 4.00 ng/ml; the endotoxin contents were < 50 EU/Dose. After vaccination to mice and guinea pigs for 7 days, all the animals were healthy, and the body weight of each mouse increased. The potencies and water contents were stable in 37±1 °C during 4 weeks, whilst the potencies were relatively constant in 2-8 °C during 4 months. This result indicated that HDCV developed by microcarrier technology in the bioreactor possessed the persistent immunogenicity and stable quality.

References


