Journal of Virological Methods 206 (2014) 89–94

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

A time-resolved fluoroimmunoassay for the quantitation of rabies virus nucleoprotein in the rabies vaccine

Guanfeng Lin[a], Hong Huang[a], Tiancai Liu[a], Chunhui He[b], Jianqing Liu[b], Shaolang Chen[a], Jingyuan Hou[a], Zhiqi Ren[a], Wenqi Dong[a], Yingsong Wu[a,*]

[a] *Institute of Antibody Engineering, School of Biotechnology, Southern Medical University, Guangzhou, China*
[b] *Guangzhou Promise Biologics Co., Ltd, No. 1 Wanbao North Street, Panyu District, Guangzhou, China*

Article history:
Received 28 February 2014
Received in revised form 30 May 2014
Accepted 3 June 2014
Available online 10 June 2014

Keywords:
Rabies virus
Vaccine
Nucleoprotein
Time-resolved fluoroimmunoassay

A B S T R A C T

Sensitive, precise and rapid detection tests are needed in the quality control of rabies vaccine for rabies virus nucleoprotein. Previous studies for quantitation of rabies virus nucleoprotein focused on enzyme-linked immunosorbent assay (ELISA). A novel immunoassay for rapid determination of rabies virus nucleoprotein in rabies vaccine was first established by time-resolved fluoroimmunoassay (TRFIA). Based on a sandwich-type immunoassay format, analytes in samples were captured by one monoclonal antibody coating in the wells and "sandwiched" by another monoclonal antibody labeled with europium chelates. The immunocomplex was retained after washing, and then adopted treatment with enhancement solution; fluorescence was then measured according to the number of europiumions dissociated. Levels of the rabies virus nucleoprotein were measured in a linear range (5–2500 mEU/mL) with a lower limit of quantitation (0.95 mEU/mL) under optimal conditions. The repeatability, recovery, and linearity of the immunoassay were demonstrated to be acceptable. The correlation coefficient of nucleoprotein values obtained by novel TRFIA method and ELISA method was 0.981. These results showed good correlation and confirmed that this sensitive, precise and rapid TRFIA was feasible and could be more suitable for the quality control in the process of rabies vaccine production than ELISA.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Rabies is an endemic, fatal but preventable zoonotic disease. It is caused by rabies virus, a member of the *Lyssavirus* genus of the *Rhabdoviridae* family. Rabies occurs in more than 150 countries and territories. Asia, with an estimated 56% of the 55,000 human rabies deaths worldwide, has 31,000 deaths per year (Dodet and Asian Rabies Expert, 2007). Most of these are in children. This is a very heavy toll. China has the second highest incidence of rabies after India (Meng et al., 2010; Ming et al., 2010). Prevention and post-exposure therapy require safe and efficacious vaccines. Vaccination is considered one of the most viable and important methods for the prevention of rabies by way of preexposure prophylaxis in high-risk groups, postexposure prophylaxis in contact groups, and preexposure prophylaxis in pet animals that are at risk due to possible contacts with rabid animals (Yokomizo et al., 2007; Nimmagadda et al., 2010). Every year, more than 15 million people worldwide receive a post-exposure vaccination to prevent the disease - this is estimated to prevent hundreds of thousands of rabies deaths annually (WHO, 2005). The viral genome of rabies virus produces five monocistronic mRNAs encoding the nucleoprotein, phosphoprotein, matrix protein, transmembrane glycoprotein and the viral RNA-dependent RNA polymerase (Wunner et al., 1988). Rabies virus nucleoprotein is one of the major components in rabies vaccine, which can contribute to the induction of neutralizing antibodies against rabies to prevent rabies by vaccination (Wiktor et al., 1973; Fu et al., 1991; Yin et al., 2013).

Antigenic analysis of a wide variety of street rabies virus and related *Lyssavirus* strains has shown that rabies virus nucleoprotein antigen is genetically and antigenically more conserved than glycoprotein (Drings et al., 1999). It contains a conserved linear epitope that is antigenically conserved to a much greater extent among most rabies virus strains, and therefore, is a better diagnostic antigen for use in a wide variety of hosts (Dietzschold et al., 1987; Mannen et al., 1991; da Cruz et al., 2001). Monoclonal antibodies against this linear epitope of nucleoprotein may have the potential to recognize native rabies virus nucleoprotein, and

Abbreviations: TRFIA, time-resolved fluoroimmunoassay; ELISA, enzyme-linked immunosorbent assay; mEU/ml, milli-enzyme units per ml; LLOQ, lower limit of quantitation; RE, relative error; MAb, monoclonal antibody.

* Corresponding author. Tel.: +86 20 62789355; fax: +86 20 37247604.
E-mail address: wg@fimmu.com (Y. Wu).

http://dx.doi.org/10.1016/j.jviromet.2014.06.002
0166-0934/© 2014 Elsevier B.V. All rights reserved.

expression of the nucleoprotein gene of rabies virus has successfully been used as a diagnostic reagent (He et al., 2006; Lv et al., 2012). Previous studies for quantitation of rabies virus nucleoprotein focused on enzyme-linked immunosorbent assay (ELISA), and just a few publications had described the detection of rabies virus nucleoprotein with ELISA (Montano-Hirose et al., 1995; Katayama et al., 1999; Xu et al., 2007). However, sensitive, precise and rapid detection tests are needed in the quality control of rabies vaccine for rabies virus nucleoprotein. Time-resolved fluoroimmunoassay (TRFIA) using europium chelates (Eu^{3+}) as the labels was used as an 'ideal' immunoassay method when it was first reported by Lovgren et al. (1984), and had been noticed as a highly sensitive method and employed in numerous applications in the biomedical sciences (Hemmila et al., 1984; Hemmila, 1985; Kricka, 1994; Dickson et al., 1995; Hou et al., 2012, 2013; Lin et al., 2013). Compare with ELISA, fluorescence immunoassay has higher sensitivity, extremely wider effective detection range and excellent repeatability (Pei et al., 2013). We herein first report the development of a novel TRFIA to replace ELISA, which was designed specifically as a sensitive, precise and rapid detection assay for quality control in the process of rabies vaccine production. Thus, the purpose of the present study was to develop a novel TRFIA and test its application for the quantitation of rabies virus nucleoprotein. This study involved measurement of parameters, such as repeatability, recovery, linearity and feasibility.

2. Materials and methods

2.1. Virus, plasmids, cells, mice and samples

The CTN strain of rabies virus was kindly provided by Guangzhou Promise Biologic Products (Guangzhou, China). Sp2/0 cell and plasmid pET-28a (+) were stored at Institute of Antibody Engineering, School of Biotechnology, Southern Medical University (Guangzhou, China). The BALB/c mice were obtained from Experimental Animal Center, Southern Medical University (Guangzhou, China). All samples were kindly provided by Guangzhou Promise Biologics (Guangzhou, China) and were stored at −70 °C. Concentrations of rabies virus nucleoprotein were measured by an ELISA (Xu et al., 2007).

2.2. Materials and instrumentation

Anti-nucleoprotein MAb (C01630M) was obtained from Meridian Life Science (Memphis, TN, USA). Restriction endonucleases, PCR enzymes, *E. coli* DH5α and BL21 competent cells were obtained from Takara Biotechnology (Dalian, China). Tris, IPTG and Triton X-100 and bovine serum albumin (BSA) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Transparent 96-well microtitration strips were purchased from Nunc (Roskilde, Denmark). A sepharose CL-6B column was obtained from the Pharmacia Company (Chalfont St Giles, UK). The DEAE-Sephadex A-50 resin was obtained from GE Healthcare (Fairfield, CT, USA). The Victor^3 1420 multilabel counterand and Eu^{3+}-labeling kit were products of PerkinElmer WALLAC (Turku, Finland). Other chemicals and reagents used were of analytical grade.

2.3. Reagent solutions

Labeling buffer contained 50 mM Na_2CO_3–$NaHCO_3$ (pH 8.5), and 155 mM NaCl. Elution buffer contained Tris-buffered saline (TBS: 50 mM Tris–HC1 pH 7.8, 0.9% NaC1), with 0.05% NaN_3. Coating buffer contained 50 mM of Na_2CO_3–$NaHCO_3$ buffer (pH 9.6). Blocking solution contained 50 mM of Na_2CO_3–$NaHCO_3$ buffer (pH 9.6) and1% BSA. Standard buffer contained 50 mM Tris–HCl (pH 7.8), 0.2% BSA and 0.1% NaN_3. Assay buffer contained 50 mM TBS with 0.02% BSA, 0.05% NaN_3 and 0.05% Tween-20. Washing solution was TBS with 0.2% Tween-20, and 0.05% NaN_3. Enhancement solution was 100 mM acetate–phthalate buffer (pH 3.2) containing 0.1% Triton X-100, 15 μM β-naphthoyltrifluoroacetate (β-NTA), and 50 μM tri-*n*-octylphosphine oxide.

2.4. Expression of rabies virus N gene in E. coli and purification the nucleoprotein

Total rabies virus RNA was extracted from samples using Viral RNA Kit (Omega Bio-Tek, Norcross, GA, USA), according to the manufacturer's instructions. The cDNA was amplified from the rabies virus RNA with random 6 primers by RT-PCR. The complete length of nucleoprotein gene of the CTN strain of rabies virus was amplified from cDNA by PCR using a pair of specific primers (forward, 5′-ATGAATTCATGGATGCCGA-CAAGATTGTATT-3′; reverse, 5′-TAAGCTTTGAGTCACTCGAATATGTCTTGTT-3′) designed according to the relevant sequences from GenBank (accession no. AF367863) and including restriction endonuclease cleavage sites for *EcoR* I (5′-G|AATTC-3′/3′-CTTAA|G-5′) and *Hind* III (5′-A|AGCTT-3′/3′-TTCGA|A-5′). The PCR product was cloned into the pET-28a (+) prokaryotic expression plasmid to obtain plasmid pET-28a/N. The target gene was then expressed in *E. coli* BL21 competent cells by induction with IPTG and the induction time was optimized to ensure maximal expression of the target protein. The antigenicity of the resulting recombinant nucleoprotein was confirmed by western blotting analysis.

2.5. Animal immunization and anti-nucleoprotein antibody detection by indirect ELISA

As per the guidelines of the Institutional Animal Care and Use Committee (IACUC), Southern Medical University, for prevention and control rabies, a simplified immunization procedure was adopted for the BALB/c mice (Howard and Kaser, 2007). Purified recombinant rabies virus nucleoprotein was dissolved in 200 μL 10 mM phosphate-buffered saline (PBS, pH 7.2), and the solution was injected into the celiac of each mouse. For the initial immunization and first and second booster immunizations, 75 μg purified recombinant nucleoprotein was mixed with 100 μL of complete Freund's adjuvant. For the last booster, 100 μg of recombinant nucleoprotein was used. The interval between any two ordinal immunizations was three weeks. The monoclonal antibodies (MAbs) against rabies nucleoprotein were prepared at the Institute of Antibody Engineering, School of Biotechnology, Southern Medical University (Guangzhou, China). Briefly, the spleen cells of BALB/c mice immunized with purified recombinant nucleoprotein were fused with Sp2/0 cells. The resulting hybridomas were screened by indirect ELISA to select those secreting MAbs with the highest affinity for rabies virus nucleoprotein. Positive hybridomas were ultimately propagated in vitro, and then used for the preparation of ascites. The MAbs were purified from ascites according to standard protocol using DEAE–Sephadex A-50 resin.

2.6. Antibody labeling

Anti-nucleoprotein MAb (F022) was labeled with Eu^{3+}-chelates. Briefly, F022 was gently mixed in 200 μL of labeling buffer with 500 μg of Eu^{3+}-chelates in 100 μL of the same buffer. After an 18 h incubation with continuous gently shaking at room temperature, free Eu^{3+}-chelates and aggregated MAb were separated from Eu^{3+}-MAb conjugates using a 1 cm × 40 cm column packed with sepharose CL-6B (lower 20 cm), eluted with a descending elution buffer, and collected (1.0 mL/fraction). The concentration of Eu^{3+}-conjugates in collected fraction was measured by fluorescence, and diluted with an enhancement solution (1:1000). The

Download English Version:

https://daneshyari.com/en/article/6133548

Download Persian Version:

https://daneshyari.com/article/6133548

Daneshyari.com