Vaccine xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

Cross-protective efficacy of engineering serotype A foot-and-mouth disease virus vaccine against the two pandemic strains in swine

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ARTICLE INFO

Article history:
Received 1 June 2015
Received in revised form
15 September 2015
Accepted 16 September 2015
Available online xxx

Keywords:
Foot-and-mouth disease virus
ASIA topotype
Engineering foot-and-mouth disease virus
Protective efficacy

ABSTRACT

Foot-and-mouth disease (FMD) is a highly contagious vesicular disease that affects domestic and wild cloven-hoofed animals worldwide. Recently, a series of outbreaks of type A FMDV occurred in Southeast Asian countries, China, the Russia Federation, Mongolia, Kazakhstan and South Korea. The FMD virus (A/GDMM/CHA/2013) from China's Guangdong province (2013) is representative of those responsible for the latest epidemic, and has low amino acid identity (93.9%) in VP1 protein with the epidemic strain A/WH/CHA/09 from Wuhan, China in 2009. Both of isolates belong to the Sea-97 genotype of ASIA topotype. Therefore, the application of a new vaccine strain with cross-protective efficacy is of fundamental importance to control the spread of the two described pandemic strains. A chimeric strain rA/P1-FMDV constructed by our lab previously through replacing the P1 gene in the vaccine strain O/CHA/99 with that from the epidemic stain A/WH/CHA/09, has been demonstrated to exhibit good growth characteristics in culture, and the rA/P1-FMDV inactivated vaccine can provide protection against epidemic strain A/WH/CHA/09 in cattle. However, it is still unclear whether the vaccine produces efficient protection against the new pandemic strain (A/GDMM/CHA/2013). Here, vaccine matching and pig 50% protective dose (PD_{50}) tests were performed to assess the vaccine potency. The vaccine matching test showed cross-reactivity of sera from full dose vaccine vaccinated pigs with A/WH/CHA/09 and A/GDMM/CHA/2013 isolates, with average r_1 values of 0.94 ± 0.12 and 0.68 ± 0.06 ($r_1 \geq 0.3$), which indicates that the rA/P1-FMDV vaccine is likely to confer good cross-protection against the two isolates. When challenged with two pandemic isolates A/WH/CHA/09 and A/GDMM/CHA/2013 strain, the vaccine achieved 12.51 PD_{50} and 10.05 PD_{50} per dose (2.8 μg), respectively. The results indicated that the rA/P1-FMDV inactivated vaccine could protect pigs against both A/WH/CHA/09 and A/GDMM/CHA/2013 pandemic isolates.

1. Introduction

Foot-and-mouth disease (FMD), is an acutely contagious disease affecting pigs, cattle, sheep, goats, and other cloven-hoofed animals which has grave economic consequences. There is a great deal of genetic variation among the seven serotypes of FMDV (A, Asia 1, C, O, and South African Territories (SAT) 1–3) [1–5]. There are many serotype A FMDV outbreaks, but lack of efficient vaccine against these epidemic strains. Many of the serotype A strains evolved, independently, in isolated geographic regions, leading to genetic and antigenic variation among the subtypes. Cross-protection between strains of serotype A may be incomplete [6]. The first report of serotype A in China was January of 2009, near Wuhan, Hubei province. Occurrences of FMDV serotype A infections were reported in nine other areas within the Chinese mainland during 2009–2010 [7]. However, 2 years after the outbreaks of the first serotype A strain, a new strain (A/GDMM/CHA/2013) was introduced into China from Southeast Asia, and was first reported in Maoming, Guangdong province in February 2013. This new strain was subsequently identified in the inland China provinces (March 2013), in the Russian Federation (March 2013), Kazakhstan (July 2013), Mongolia (August 2013) South Korea (July 2014) and Taiwan (April 2015). These outbreaks led to the severe economic loss, and their strains are still widely prevalent in the above geographic

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http://dx.doi.org/10.1016/j.vaccine.2015.09.055
0264-410X/

regions, continue to threaten FMD-free regions and have attracted a great deal of global interest. The unusual spread of the FMD strain in these counties became one of the most serious epidemic situations in recent times, and raised concerns about the possibility of disease spread within and outside the region, as happened during the FMDV O/MYA/98 pandemic of 2010–2013 [8]. The re-emerging FMDV strain in China's Guangdong province (2013) has low amino acid identity (93.9%) with the epidemic strain, A/WH/CHA/09 from Wuhan, China in 2009, and both of FMDV strains belong to the Sea-97 genotype of ASIA topotype. During 2009–2010, in order to overcome issues with selection and adaptation of vaccine strain and to get better vaccine candidates from field isolates, using reverse genetics technology, we substituted the P1 gene from field isolate A/WH/CHA/09 for P1 gene in a cDNA clone of a vaccine strain (O/CHA/99), creating a chimera (rA/P1-FMDV). The chimera exhibited better growth characteristics in culture than the isolate A/WH/CHA/09 and its inactivated vaccine had good protective effect against epidemic strain A/WH/CHA/09 in cattle [7]. However, whether the vaccine would protect against the new pandemic strain (A/GDMM/CHA/2013) is still unclear.

The present study describes pig vaccinations with the rA/P1-FMDV inactivated vaccine and, within 7 days, that most of the pigs displayed a humoral immune response. FMDV-neutralizing antibody test was used to determine the vaccine matching relationship (r_1) values for rA/P1-FMDV against A/WH/CHA/09 and A/GDMM/2013. The cross-protective efficacy of inactivated, oil-emulsified vaccines prepared from the chimera against isolates of the two pandemic strains was evaluated. Viral RNAs and nonstructural protein (NSP) 3ABC antibodies were also tested to evaluate the clinical infections in pigs by rRT-PCR and the commercially available 3ABC-I-ELISA kit respectively.

2. Materials and methods

2.1. Cells and viruses

BHK-21 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; HyClone), supplemented with 10% fetal bovine serum (FBS; HyClone), 100 U/mL penicillin and 100 mg/mL streptomycin at 37 °C in a 5% CO_2 atmosphere. A/WH/CHA/09 was isolated from Wuhan, China in February 2009 and A/GDMM/CHA/2013 was isolated from Maoming, Guangdong province, China in February 2013. The pig median infected dose (PID_{50}) of A/GDMM/CHA/2013 passaged in suckling mice were evaluated as 5.0 $\log_{10}$/2 mL using the method described by Li et al. [9]. The rA/P1-FMDV was rescued by reverse genetics technology in our laboratory previously [7]. The same batch of the viruses passaged in suckling mice was used in this study.

2.2. RNA extraction and real-time quantitative PCR (qPCR)

Total RNA was extracted from tissue samples of A/WH/CHA/09 and A/GDMM/CHA/2013 using the RNeasy kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. From these RNA samples, cDNAs were synthesized. Two μg of the extracted RNAs were used as templates for cDNA synthesis. Reverse transcription (RT) reactions were carried out using random hexamer primers and M-MLV reverse transcriptase (Life Technologies, Carlsbad, CA, USA). The viral RNAs were detected by qPCR assay that had been previously established [7,10]. The results from all samples were analyzed using Stratagene® MxPro™ QPCR software and a CT value was assigned to each reaction as described previously [10]. For FMDV viral RNA detection, the sample with a CT value ≤35 was deemed as positive [7].

2.3. Antigen and vaccine production

Antigen and vaccine production were performed according to the procedure described by Zheng et al. [7]. Briefly, BHK-21 cells were cultured in roller bottles (1500 cm^2). Supernatants were collected from BHK-21 infected cultures at the 12th passage of rA/P1-FMDV. These supernatants were clarified and used to inoculate the roller bottle cultures. When 100% cytopathic effect was apparent, the virus was harvested. 10% of the clarified cell culture supernatants were kept as a live virus and stored at −70 °C for later use; and the rest part was inactivated by BEI and purified by a sucrose density gradient to prepare the vaccine.

The water-in-oil-in-water (WOW) rA/P1-FMDV vaccines were prepared containing 2.8 μg of BEI-inactivated 146S FMDV antigen purified by a sucrose density gradient centrifugation [11]. The content of the purified antigen was confirmed by OD260 measurement. The oil adjuvant (Montanide ISA 206 (Seppic)) was mixed (50:50) with the aqueous phase.

2.4. Protective dose 50% (PD_{50}) test and detection of FMDV-specific and neutralizing antibody in pigs

A total of 36 six-week-old pigs were randomly allocated to eight groups (Table 1). The pigs in groups 1 and 5 were immunized intramuscularly in the neck with one dose of vaccine (2 mL, 2.8 μg), pigs in groups 2 and 6 with 1/3 dose of vaccine, and pigs in group 3 and 7 with 1/9 dose of vaccine. The unvaccinated pigs, in groups 4 and 8, served as the negative controls. On the vaccination date (day 0) and each 7 days post-vaccination (dpv) thereafter (e.g. days 7, 14, etc.), serum samples were collected from each pig, for 4 weeks. Vaccine potency was evaluated by a method similar with the vaccine potency test protocol for cattle, which is described in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of the Office International Des Epizooties (OIE). Two potency tests were performed in which the three vaccinated groups of 5 pigs were administered different doses (full dose, 1/3 dose or 1/9 dose) of vaccine prepared from the rA/P1-FMDV strain. At 28 dpv, the pigs in groups 1, 2, 3 and 4 were challenged by intramuscular inoculation in the neck behind the ear with 1000 SID_{50} of A/WH/CHA/09. The pigs in groups 5, 6, 7 and 8 were challenged by intramuscular inoculation in the neck behind the ear with 1000 SID_{50} of A/GDMM/CHA/2013 at 28 dpv. On 0, 7, 14, 21 and 28 dpv, serum titers of FMDV-specific antibody against rA/P1-FMDV were determined, according to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2009), with a liquid-phase blocking ELISA (LPBE). Meanwhile, on 28 dpv, serum titers of FMDV-neutralizing antibody against rA/P1-FMDV were determined, according to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2009), with a Virus Neutralization Test (VNT).

2.5. Vaccine matching

Vaccine matching was performed by a VNT, comparing the reactivity of pig antisera against rA/P1-FMDV, A/WH/CHA/09 and A/GDMM/CHA/2013. Vaccines of normal potency are deemed unlikely to provide effective protection with an r value of less than 0.3 [12,13].

2.6. Swine challenge experiments and 3ABC antibody detection

For the challenge experiments, each group of pigs was housed in one independent house, and pigs presenting the clinical signs were separated. The pigs immunized with different doses vaccines were challenged in the neck behind the ear with the field isolated strains A/WH/CHA/09 and A/GDMM/CHA/2013. Then, daily observations were made and clinical signs were recorded of each pig for

Please cite this article in press as: Zheng H, et al. Cross-protective efficacy of engineering serotype A foot-and-mouth disease virus vaccine against the two pandemic strains in swine. Vaccine (2015), http://dx.doi.org/10.1016/j.vaccine.2015.09.055