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Evaluation of inactivated vaccines against equine group A rotaviruses by use of a suckling mouse model

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ABSTRACT

Background: Equine group A rotaviruses (RVAs) cause diarrhea in suckling foals. The dominant RVAs circulating among horses worldwide, including Japan, are G3P[12] and/or G14P[12] genotypes. Inactivated vaccines containing a G3P[12] RVA are commercially available in some countries for prevention of diarrhea caused by equine RVAs. However, there is no reported evidence whether vaccines containing a G3P [12] RVA are effective against G14P[12] RVAs or whether using a G14P[12] RVA results in a more effective vaccine. This study used a suckling mouse model to evaluate the effectiveness of inactivated vaccines containing G3P[12] (G3 vaccine) or G14P[12] (G14 vaccine) RVAs against G3P[12] and G14P[12] RVAs.
Methods: Female mice were inoculated twice with G3 or G14 vaccines, and were then mated. After parturition, suckling mice were challenged with one of either two G3P[12] RVAs, two G14P[12] RVAs, or one G13P[18] RVA. After virus inoculation, suckling mice were observed for diarrhea, and the incidence rates of diarrhea in the vaccinated groups were compared with those in the non-vaccinated groups.
Results: Following G3P[12] RVA challenge, suckling mice in the G3 and G14 vaccinated groups had significantly lower rates of diarrhea incidence than did those in the non-vaccinated group, and the rates in the G3 vaccinated group tended to be lower than in the G14 vaccinated group. Following G14P[12] RVA challenge, suckling mice in the G14 vaccinated group had significantly lower rates of diarrhea incidence than did those in the non-vaccinated and G3 vaccinated groups. The G3 and G14 vaccines did not reduce the rate when challenged with the G13P[18] RVA.
Conclusion: The mouse model showed that the G3 and G14 vaccines were both effective against G3P[12] RVAs, and that the G14 vaccine was effective against G14P[12] RVAs. These results suggest that at least a G14 RVA strain should be included in as a vaccine strain.

1. Introduction

Equine group A rotaviruses (RVAs) are non-enveloped viruses with 11 double-stranded RNA genome segments; they belong to the genus *Rotavirus* in the family *Reoviridae* [1]. Equine RVAs are the main cause of diarrhea in suckling foals younger than 4 months old [2]. Two outer capsid proteins, VP7 and VP4, independently elicit neutralizing antibodies, induce protective immunity, and are used to classify RVAs into G (for glycoprotein) and P (for protease-sensitive) genotypes [1]. Based on this classification, the main RVAs circulating among the horse population in many countries are reported to be G3P[12] and/or G14P[12] genotypes [3–7]. In Japan, G3P[12] RVAs were predominant until the early 1990s [8] but G14P[12] RVAs were newly detected in the late 1990s [9]. In recent years, G3P[12] and G14P[12] RVAs have been co-circulating in Japan like in other countries [10]. In addition, a whole-genome classification system based on nucleotide sequences is shown by using the following formula: Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx that represents VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5 genotypes, respectively [11]. It has been reported that equine G3P[12] and G14P[12] RVAs possess a largely conserved genotype constellation (G3/G14-P[12]-I2/I6-R2-C2-M3-A10-N2-T3-E2/E12-H7) [12–15].

Inactivated vaccines have been used in some countries for the prevention of diarrhea induced by equine RVA infection. They are administered twice intramuscularly to pregnant mares, and their newborn foals obtain passive immunity via the colostrum. RVA/Horse-tc/GBR/H-2/1976/G3P[12] has been used as a vaccine strain in the United States [16], the United Kingdom, and Ireland [7,17]. In Argentina, three RVA strains (RVA/Horse-tc/GBR/H-2/1976/G3P

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[12], RVA/Simian-tc/ZAF/SA11/1958/G3P[2], and RVA/Cow-tc/USA/NCDV-Lincoln/1967/G6P[1]) have been used for vaccination [18]. In Japan, the vaccine contains the strain RVA/Horse-tc/JPN/HO-5/1982/G3P[12] (HO-5/G3) on the basis of the epidemic situation until the early 1990s, and it has been commercially available since 2001 (Nisseiken Co., Ltd., Tokyo, Japan) [19,20]. Our previous study using vaccinated pregnant mares showed that the vaccine containing HO-5/G3 induces virus-neutralizing antibodies against not only G3 RVAs but also G14 RVAs, although titers of antibodies against G14 RVAs were lower than the titers of antibodies against G3 RVAs [21]. To our knowledge, there is no report so far showing whether a vaccine containing a G3 RVA is effective against a G14 RVA or whether a G14 RVA should be used to obtain a more effective vaccine.

To evaluate the Japanese vaccine, an experimental challenge study had been conducted using suckling foals [19], but studies using foals are extremely laborious. Suckling mice also develop diarrhea when inoculated with equine RVAs [22], and challenge studies using mice are far less laborious than studies using foals. Therefore, suckling mice would be a potentially good model to evaluate the effectiveness of equine RVA vaccines. The purpose of this study was to evaluate the effectiveness of equine RVA vaccines against G3 and G14 RVAs by using a mouse model.

2. Materials and methods

2.1. Mice

All mice used in this study were Slc:ddY mice purchased from Japan SLC, Inc. (Shizuoka, Japan) because it has been reported that Slc: ddY mice had diarrhea by inoculation of pigeon and simian RVAs [23]. Pregnant mice were obtained at day 14 of gestation and allowed to give birth at our laboratory; the litters from some of these mice ($n = 20$) were used to determine 50% diarrhea-inducing doses (DD_{50}) for each of the five RVAs, and the other pregnant mice ($n = 29$) were used as non-vaccinated control mice. Seven-week-old non-pregnant mice ($n = 29$ for each vaccine) were obtained for use as the vaccinated groups. Additionally, 12-week-old male mice were used for mating; the ratio of males to females in a cage was 1:2 or 1:3. All experimental procedures were approved by the Animal Care Committee of the Equine Research Institute of the Japan Racing Association.

2.2. Viruses

Five equine RVA strains were used as challenge viruses. These were HO-5/G3 [8], RVA/Horse-tc/JPN/No.1/2010/G3P[12] (No.1/2010/G3) [21], RVA/Horse-tc/JPN/JE77/1997/G14P[12] (JE77/G14) [9], RVA/Horse-tc/JPN/No.50/2010/G14P[12] (No.50/2010/G14) [21], and RVA/Horse-tc/GBR/L338/1991/G13P[18] (L338/G13) [24]. Strains other than No.50/2010/G14 were propagated in MA-104 cells, and No.50/2010/G14 was propagated in Caco-2 cells as described previously [21]. The culture medium was composed of Eagle's minimum essential medium containing 10% tryptose phosphate broth, 0.05% yeast extract, 0.05% glucose, 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B [25]. After propagation, viruses were concentrated by ultrafiltration membranes (Amicon Ultra-15 and Centricon Plus-70, Ultracel-PL 100 KDa; Merck Millipore, Darmstadt, Germany) and used to induce diarrhea in suckling mice.

2.3. Determination of DD_{50}

To determine the DD_{50} of the five concentrated equine RVA strains, 50 μL of serially 10-fold diluted viruses (nil, 10^{-1}, 10^{-2}, and 10^{-3} dilution) was inoculated by oral gavage into 3- or 4-day-old suckling mice. One litter of suckling mice was used with respect to each virus dilution. Inoculated mice were observed daily at 1, 2, and 3 days post-inoculation (dpi) for diarrhea by pressing the abdomen gently. DD_{50} was calculated by the method of Reed and Muench [26] from the number of mice showing diarrhea,

2.4. Preparation of inactivated vaccines

Commercially available vaccine containing HO-5/G3 (Equine Rotavirus Disease Vaccine (Inactivated), Lot no. 15; Nisseiken Co., Ltd) (G3 vaccine) and a vaccine containing JE77/G14 of our own making (G14 vaccine) were used. Aluminum (III) chloride hexahydrate was employed as adjuvant in both vaccines. Mice received 200 μL of the commercial G3 vaccine at each vaccination. Two batches of JE77/G14 (4.3×10^6 fluorescing focus units (FFU)/36 mL and 1.8×10^7 FFU/36 mL) were inactivated by 0.2% formalin at 37 °C for 69–72 h. Inactivated virus was dialyzed with phosphate buffered saline by using Slide-A-Lyzer G2 Dialysis Cassettes, 10 K molecular weight cut off (Thermo Fisher Scientific, Waltham, MA, USA) for removal of formalin. Protein concentrations of solutions containing inactivated JE77/G14 were measured by using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA), and 20 μg/head of inactivated JE77/G14 was utilized for vaccination.

2.5. Immunization of female mice, mating, and inoculation of suckling mice with viruses

The experimental schedule in this study mimicked the Japanese vaccine program in horses (Fig. 1). Female mice ($n = 29$ for each vaccine) were intraperitoneally immunized with the G3 or G14 vaccine twice at an interval of 2 weeks. From the 6th day after the second vaccination, the immunized female mice were kept with male mice for 8 days for mating. For the non-vaccinated control group we used purchased pregnant mice ($n = 29$). After parturition, five litters of 3- to 4-day-old suckling mice were orally inoculated with each equine RVA strain ($3 \times DD_{50}$, 50 μL/head; see Results and Discussion section), and four litters were given culture medium (50 μL/head) as negative control. Inoculated mice were observed at 1, 2, and 3 dpi for diarrhea by pressing the abdomen gently.

2.6. Virus neutralization test

Blood samples were collected from the dams on the last day of clinical observation of suckling mice. Serum samples were diluted 1:40, and then twofold serial dilutions of serum were used in virus neutralization tests. The virus neutralization test was performed in MA-104 cells by the fluorescent focus neutralization test, as described previously [27]. Virus-neutralizing antibody titers were expressed as the reciprocal of the highest serum dilution that resulted in an 80% or greater reduction in fluorescent foci. Strains HO-5/G3, JE77/G14, and L338/G13 were used as reference strains.

2.7. Statistical analysis

All analyses were performed with the R statistical package (version 3.4.3; R Foundation for Statistical Computing, Vienna, Austria) to evaluate the effectiveness of vaccines against equine RVAs. Multiple pairwise comparisons were performed to determine *P*-values that were then adjusted according to the Benjamini–Hochberg false discovery rate procedure [28]. Values with an adjusted *P*-value of <0.05 were considered statistically significant.