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Coinjection of a vaccine and anti-viral agents can provide fast-acting protection from foot-and-mouth disease

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ABSTRACT

Foot-and-mouth disease (FMD) is the cause of an economically devastating animal disease. With commercial inactivated FMD vaccines, the protection against FMD virus (FMDV) begins a minimum of 4 days post vaccination (dpv). Therefore, antiviral agents could be proposed for rapid protection and to reduce the spread of FMDV during outbreaks until vaccine-induced protective immunity occurs. In previous studies, we have developed two recombinant adenoviruses that simultaneously express porcine interferon-α and interferon-γ (Ad-porcine IFN-αγ) and multiple siRNAs that target the non-structural protein-regions of FMDV (Ad-3siRNA), and we have shown that the combination of the two antiviral agents (referred to here as Ad combination) induced robust protection against FMDV in pigs. In an attempt to provide complete protection against FMDV, we co-administered Ad combination and the FMD vaccine to mice and pigs. In the C57BL/6 mice model, we observed rapid and continuous protection against homologous FMDV challenge from 1 to 3 dpv—the period in which vaccine-mediated immunity is absent. In the pig experiments, we found that most of the pigs (five out of six) that received vaccine + Ad combination and were challenged with FMDV at 1 or 2 dpv were clinically protected from FMDV. In addition, most of the pigs that received vaccine + Ad combination and all pigs inoculated with the vaccine only were clinically protected from an FMDV challenge at 7 dpv. We believe that the antiviral agent ensures early protection from FMDV, and the vaccine participates in protection after 7 dpv. Therefore, we can say that the combination of the FMD vaccine and effective antiviral agents may offer both fast-acting and continuous protection against FMDV. In further studies, we plan to design coadministration of Ad combination and novel vaccines.

1. Introduction

Foot-and-mouth disease (FMD) is an acute contagious disease affecting cloven-hooved animals, such as cows, pigs, sheep, goats, and deer. It induces fever, lameness, and vesicles on the mouth, tongue, snout, teats, and feet (Alexandersen et al., 2003; Moraes et al., 2007). FMDV belongs to the *Aphthovirus* genus of the *Picornaviridae* family; it has a single-stranded, plus-sense RNA genome. The virus consists of seven serotypes: A, O, C, Asia1, and South African Territories 1, 2, and 3 (SAT1, SAT2, and SAT3). There is little to no cross immunity between the different serotypes (Alexandersen and Mowat, 2005; Moraes et al., 2007).

FMD commercial vaccines are derived from inactivated whole-viruses, manufactured either as aqueous or oil-based formulations with aluminum hydroxide or saponin or as single or double emulsions for oil adjuvants (Doel, 2003). The inactivated vaccines are currently used around the world, including Asia, to prevent transmission of FMDV. It is an inactivated whole virion mixed with an oil-based adjuvant (Grubman and Baxt, 2004; Saeed et al., 2015). This type of vaccine requires a minimum of 4 days after vaccination for successful protection against FMDV in cases of indirect aerosol FMDV infection (Cox and Barnett, 2009). When emergency

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Abbreviations

CPE	Cytopathic effect
DPC	Days post-challenge
DPV	Days post vaccination
FMD	Foot-and-mouth disease
FMDV	Foot-and-mouth disease virus
IFN	Interferon
IP	Intraperitoneal
SD	Standard deviation
siRNAs	Small interfering RNAs
RT-PCR	Reverse transcriptase polymerase chain reaction
$TCID_{50}$	50% tissue culture infective dose
LD_{50}	50% lethal dose

vaccinations must be administered in response to an FMD outbreak in an FMD-free country, the time taken for vaccination to become effective is too long (Borrego et al., 2013; Golde et al., 2005). Therefore, it is necessary to provide rapid protection against FMDV until appearance of vaccine-induced protective immunity. Interferons, chemical compounds, or small interfering RNAs (siRNAs) have been reported to induce rapid protection from FMD (Chen et al., 2004; Chinsangaram et al., 2003; Goris et al., 2007; Jeong et al., 2015; Kim et al., 2008; Moraes et al., 2007; Perales et al., 2009). We have also developed two recombinant adenoviruses that simultaneously express porcine interferon-α and interferon-γ (Ad-porcine IFN-αγ) and multiple siRNAs that target the non-structural proteins-regions 2B and 3C of FMDV (Ad-3siRNA) (Kim et al., 2010, 2014). In a recent study, we showed that the combination treatment of the two antiviral agents Ad-porcine IFN-αγ and Ad-3siRNA (referred to here as Ad combination) with different antiviral mechanisms induces robust protection against FMDV in pigs. The combination approach has been proven to be fast-acting, effective against seven FMDV serotypes, and advantageous to overcoming the mechanisms of resistance of FMDV (Kim et al., 2015); however, we also found that the antiviral effect of Ad combination was time-dependent and significantly decreased at 7 days after injection. Therefore, our aim was to test a combination of a vaccine and Ad combination for fast-acting and consistent protection. The combination of a vaccine and an antiviral agent has been reported to induce more rapid and efficient protection in studies on the human hepatitis C virus (HCV) and smallpox virus (Berhanu et al., 2015; Scott et al., 2015). In an FMDV study, protection against FMDV has been reported in cattle that were injected with an FMD subunit vaccine and an antiviral agent, IFN-α, and were challenged with FMDV at 1 day post vaccination (dpv) (Grubman, 2005).

In the present study, we show that the use of Ad combination with a conventional FMD inactivated vaccine exerts rapid and consistent protective effects against FMDV. First, we used a mouse model to examine the change of protection rate as a function of time (days post vaccination; dpv), and then we determined whether coadministration of the inactivated FMD vaccine and Ad combination has synergistic protective effects and how the protective role of each component in the combination changes with time after coinoculation. In pig experiments, we tested the synergistic effects of the coadministration of Ad combination with the vaccine 1, 2, and 7 dpv. We observed rapid anti-FMDV effects of Ad-combination at 1 and 2 dpv and a protective anti-FMDV effect of the vaccine at 7 dpv. We also monitored changes in cytokine and antibody levels in pigs injected with Ad combination, the vaccine, or a mixture of the two for 7 days after injection.

2. Materials and methods

2.1. Cells, viruses, and titrations

Human embryo kidney (HEK) 293 cells and porcine kidney (LF-PK) cells (LaRocco et al., 2015) were cultured in Dulbecco's modified Eagle's medium (DMEM) and supplemented with 10% fetal bovine serum (FBS; pH 7.4) at 37 °C in a 5% CO_2 incubator. The HEK 293 cells, the adenovirus packaging cell expressing human adenovirus type5 E1 DNA, were used to identify the production and titers of the recombinant adenoviruses (Ad-porcine IFN-αγ and Ad-3siRNA). The development and production of the recombinant adenoviruses was consistent with that of our previous experiments (Kim et al., 2010, 2015). LF-PK cells were used for virus neutralization test and virus titrations of the FMDV. The virus titers were calculated using the Reed and Muench method at a 50% tissue culture infective dose ($TCID_{50}$) (Reed and Muench, 1938). FMDV Asia1/Shamir89 (GenBank accession number GU582125) was used for the viral challenge in mice experiments. FMDV O/Andong/SKR/2010 (GenBank accession number KC503937) was used for the viral challenge in a pig experiment. O/Andong/SKR/2010 was used as a virus for the virus neutralization test.

2.2. FMDV challenge after vaccination or application of antiviral agents in C57BL/6 mice

Eight weeks old C57BL/6 female mice supplied by the Orient Co. Ltd (Republic of Korea) were used for this experiment. The animals were kept in the Animal and Plant Quarantine Agency (APQA) and were used with the approval of the Animal Care and Use Committee. The experiment using mice as subjects was divided into two independent experiments. Both experiments included the control group that was injected with the phosphate buffered saline (PBS) at a 0.1 ml dose one day before the challenge. The mice were challenged by intraperitoneal (IP) injection with 0.1 ml of Asia 1/Shamir89 FMDV strain at 100 LD_{50} (50% of a lethal dose), and the actual dose was 10^5 $TCID_{50}$. All mice were observed for 7 days after the challenge.

2.2.1. Mice experiment 1 (measurement of the beginning of protection by the vaccine in mice)

Twenty mice were divided into four groups including control group and administered intramuscularly with the high potency (>6PD_{50}) and oil adjuvant based FMD vaccine (Greencross Veterinary Product Co. LTD., Republic of Korea) or PBS. The antigen of the vaccine was produced by Merial (Lyon, France) and included the O1/Manisa/Turkey/69, A/Malaysia97, and Asia1/Shamir89 FMDV strains. The mice were inoculated with the vaccine dose equivalent to $^1/_{200}$ of a pig dose. After one, two, or three days post vaccination (dpv), the animals were challenged with FMDV.

2.2.2. Mice experiment 2 (co-administration of the vaccine and Ad combination in mice)

Forty mice were divided into four groups including the control group and were administered the Ad combination (Ad-porcine IFN-αγ and Ad-3siRNA), the vaccine, the vaccine and Ad combination, or PBS respectively. The vaccine was used at the same dose as the mice in the first experiment. The Ad-porcine IFN-αγ and the Ad-3siRNA were used with 5×10^8 $TCID_{50}$ as the same titer. The groups were divided in half and challenged with FMDV at 1 or 2 dpv. The survival rate was monitored in all groups. And then, FMDV challenge experiment was performed at 2 dpv as described previously for serum collection. Twenty three sera samples were collected at 1