

AN ANTIVIRAL AGENT, T-1105 PREVENTS FROM VIRUS EXCRETION FROM PIGS INFECTED WITH PORCINOPHILIC FOOT-AND-MOUTH DISEASE VIRUS

S. Ohashi¹, K. Sakamoto^{1*}, K. Fukai¹, K. Morioka¹, R. Yamazoe¹, K. Takahashi² and Y. Furuta²

¹Exotic Diseases Research Station, National Institute of Animal Health, 6-20-1 Jousui-honcho, Kodaira, Tokyo 187-0022, Japan.

²Toyama Chemical Co., Ltd., 3-2-5 Nishishinjuku, Shinjuku-ku, Tokyo 160-0023, Japan

ABSTRACT

Introduction

It is a great challenge to control the spread of foot-and-mouth disease virus (FMDV) from the infected animal, especially pigs. We evaluated effectiveness of T-1105, one of pyrazinecarboxamide derivatives, in the virus excretion from the pigs infected with O/TAW/97, known as porcophilic strain.

Materials and methods

One hour before the virus inoculation of 106.2 TCID₅₀ of FMDV O/TAW/97, 200 mg/kg of T-1105 was orally administered to four pigs. The same dose of T-1105 was administered twice a day for 7 days. Two control pigs were all done without administration. Virus excretion in nasal swab and virus contents in plasma were examined by real-time PCR. Antibody to FMDV was measured by virus neutralization test and liquid phase blocking (LPB) ELISA.

Results

The control pigs showed the typical clinical signs. In the administered group two pigs showed no clinical sign but other two pigs formed vesicles at the limited site of the injection and the viral RNA was detected from nasal swab samples. Viremia was detected three of the four pigs at early stage of infection. But amounts of viral RNA in plasma were ten times lower than non-administered group. Both antibodies titers of LPB ELISA and the virus neutralization test were lower than those of non-administered group.

Discussion

By the oral administration of T-1105, some pigs inoculated with porcophilic FMDV created mild symptoms but the duration of viremia became shorter and the virus excretions from nasal route were nothing or minor than that in non-administered group. The antibody responses to FMDV were so low that it was considered there was no or low virus replication in the pigs. It was suggested that administration of T-1105 also controlled virus excretion from pigs infected with porcophilic FMDV.

1. INTRODUCTION

The foot-and-mouth disease (FMD) is the most contagious disease in cloven-hoofed animals, including cattle, swine, sheep and goats as well as a variety of wild animal species. FMD virus (FMDV) classified in the genus *Aphthovirus*, family *Picornaviridae*. The virus is antigenically variable and seven distinct serotypes of the virus O, A, C, Asia1 and the South African Territories types 1, 2 and 3. FMDV can spread rapidly in susceptible animal herds. In 1997, because of pig industries in Taiwan had hit by the pig-adapted strain (porcophilic strain) of FMDV, more than 4 million pigs were killed and made Taiwan losing FMD free status (Yang *et al.* 1999). Strain O/TAW/97 has been shown to have a species-specific adaptation to pigs (Dunn and Donaldson, 1997) and only caused severe clinical disease in pigs.

FMD has the severe socioeconomic impact. To control the disease there are two policies of "test and slaughter" and/or "vaccination". The countries where the FMD outbreaks occur will decide to

use either or both approaches, depending on the epidemiological situation of their outbreaks. Since the 2001 FMD outbreak in the UK, there has been changes concerning control measure in Europe which use vaccination as a means of reducing dependence on culling of animals. Though the best currently available, chemically inactivated vaccines only confer complete clinical protection against homologous challenge 7 days after vaccination and partial protection in 4 days (Golde *et al.* 2005). Therefore, the use of current FMD vaccines to induce early protection is limited and alternative/supplementary methods to rapidly reduce the spread of FMDV in outbreak situations are needed.

We evaluated effectiveness of T-1105, one of pyrazinecarboxamide derivatives, on the virus excretion from the pigs infected with O/TAW/97, known as porciphilic strain. Vaccination is the one of the gold standards for FMD control measure. Moreover, we would be able to provide new alternative option to control FMD outbreak.

2. MATERIAL AND METHODS

2.1 Compounds

T-1105 was provided by Y. Furuta of Toyama Chemical Co., Ltd. (Toyama, Japan). T-1105 was dissolved in minimum essential medium (MEM) for in vitro and vivo studies for animal studies.

2.2 Viruses and cells:

Porciphilic FMDV, strain O/TAW/97, was propagated on baby hamster kidney (BHK-21) cells. The cells were maintained in Eagle's MEM (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 0.295% tryptose phosphate broth (Difco Laboratories, Detroit, Mich.), 0.15% sodium bicarbonate, 2 mM L-glutamine, and 5% fetal bovine serum.

2.3 Animal experiment: Animal experimentation was carried out in accordance with National Institute of Animal Health guidelines for Animal experiments. Six female conventional pigs weighing 20 kg were used in this experiment. One hour before virus inoculation, the administered group (n=4) was orally administered T-1105 at a dose of 200 mg/kg/day. Administered group were challenged intradermally into the heel bulb with 106.2 TCID₅₀ of FMDV O/TAW/97. Non-administrated pigs (n=2) were treated in same manner administration of T-1105. After virus challenge, the oral administration with T-1105 at the same dose was continued twice a day for 7 days. As a control, two pigs were all done in the same manner **except** administration of the compound. The pigs were monitored daily for clinical signs, including rectal temperature. Nasal swabs and heparinized blood were collected daily for monitoring virus excretion and viremia. Serum samples were collected daily for serological assay.

2.4 RNA extraction and quantitative real-time reverse transcription (RT)-PCR

Viral RNA from plasma and saliva were extracted with a High Pure Viral RNA kit in accordance with the manufacturer's instruction (Roche Diagnostics-Boehringer Mannheim, Mannheim, Germany). For the quantification of FMDV RNA, real-time RT-PCR was carried out as described elsewhere with minor modification (Oleksiewicz *et al.* 2001). The one-step quantitative RT-PCR assay was performed using a pair of primers TaqManFMD-IRES-F 5'-CTGTCTCGTAGCGGAGCATG-3' and TaqManFMD-IRES-R 5'-GCCCCGTGGGTCCTT-3', targeting the internal ribosomal entry site region and a TaqManFMDProbe-IRES 5'-VIC-TGGCCGTGGAACTCCTCCTTG-TAMRA-3' with TaqMan One-Step RT-PCR Master Mix Reagents Kit (Applied Biosystems).

2.5 Serum neutralization test

Serum neutralizing antibodies to FMDV were measured by a micro plate assay using IB-RS-2 cells and FMDV strain O/TAW/97. Briefly, One hundred 50% tissue culture infective doses of virus were added to each serum dilution. The mixtures were incubated at 37°C for 1 h, and then IB-RS-2 cells suspended in MEM were added to each well. After incubation at 37°C for 3 days in a humidified 5% CO₂ atmosphere, the neutralizing antibody titers were expressed as the reciprocal of the highest dilution of sera that completely neutralized for 50% of the challenge virus.

2.6 Detection of antibody in sera by ELISA: A LPB ELISA was used to measure antibodies against FMDV, as described previously (Ferris *et al.* 1990).

3. RESULTS

The control pigs showed the typical clinical signs such as fever, severe vesicles on their feet within 1 to 2 days post infection. Vesicles were ruptured until 3 days post infection and pigs exhibited lameness (Table). In control pigs, viral RNA in nasal swabs were detected between 1 and 7 days post infection and viremia lasted for 3 days (Figures 1 & 2). In administered group, two pigs didn't show any clinical signs but other two pigs formed vesicles only at the limited site of the injection

and the viral RNA was detected from nasal swab samples at 2 and 3 days post infection. Viremia was detected three of the four pigs at early stage of infection (Figures 1 & 2). But amounts of viral RNA in plasma from them were ten times lower than those of control group. Both antibodies titers of LPB ELISA and the serum neutralization test were also lower than those of non-administered group (Figures 3 & 4).

4. DISCUSSION

Pyrazinecarboxamide derivatives are known to have a potent anti-RNA viral activity (Furuta *et al.* 2002, Gowen *et al.* 2007., Julander *et al.* 2007). T-705, one of the Pyrazinecarboxamide derivatives, inhibited influenza virus RNA polymerase (Furuta *et al.* 2005). T-1105, a substituted pyrazine compound, has been found to exhibit potent anti-FMDV activity in vitro and in vivo (Sakamoto *et al.* 2006). The inhibitory mechanism of T-1105 is considered to be the inhibition of FMDV RNA-dependent RNA polymerase.

Pigs are important amplifiers of FMDV because of the abundance of infectious material excreted out of the body (Sellers *et al.* 1977). Recent intensification of agricultural systems has led to a massive increase in the size and density of susceptible pig populations provides potential for large scale and rapid spread of FMDV. Our study is aimed to evaluate the efficacy of T-1105, antiviral compound, in giving inhibition to pigs from infection, virus replication, virus excretion and clinical disease.

At last EUFMD in Cyprus, we demonstrated that pigs which were administered with T-1105 developed no clinical symptoms by challenging the FMDV strain O/JPN/2000. Virus excretion from these pigs was not detected from their nasal swabs by the real-time RT-PCR assay. Although only slight increase of the both ELISA and serum neutralizing antibodies was observed, it is considered that inoculated FMDV could not or slightly replicate in the pig. These results are suggesting that T-1105 is effective antiviral agent against FMD infection in pigs.

We confirmed inhibitory effect of T-1105 in case of infection with porcophilic strain of FMDV. By the oral administration of T-1105, some pigs inoculated with porcophilic FMDV created mild symptoms but the duration of viremia became shorter and the virus excretions from nasal route were nothing or minor than that in control group. The antibody responses to FMDV were so low that it was considered there was no or low virus replication in the pigs. In infection with O/JPN/2000, T-1105 was completely inhibited viral replication in pigs. It was suggested that administration of T-1105 also controlled virus excretion from pigs infected with porcophilic FMDV. This anti-FMDV agent has advantages of an immediate effect at the early stage of the virus infection. It is considered that the use of this kind of excellent antiviral agents in FMD outbreaks of FMD free countries without vaccination can be a strong tool to control the disease and to reduce the spread of FMD outbreaks.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

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Table Effects of T-1105 clinical signs

		days post infection								
		1	2	3	4	5	6	7	8	9
T-1105 Administration group	Pig No. 2	-	+	+	+	++	+	-	-	-
	3	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-
	5	-	+	+	+	++	+	-	-	-
Unadministration (Control) group	Pig No. 1	+	+	++	++	++	++	++	++	++
	6	+	+	++	++	++	++	++	++	++

+ Creation of vesicular lesion
++ Rupture of vesicle

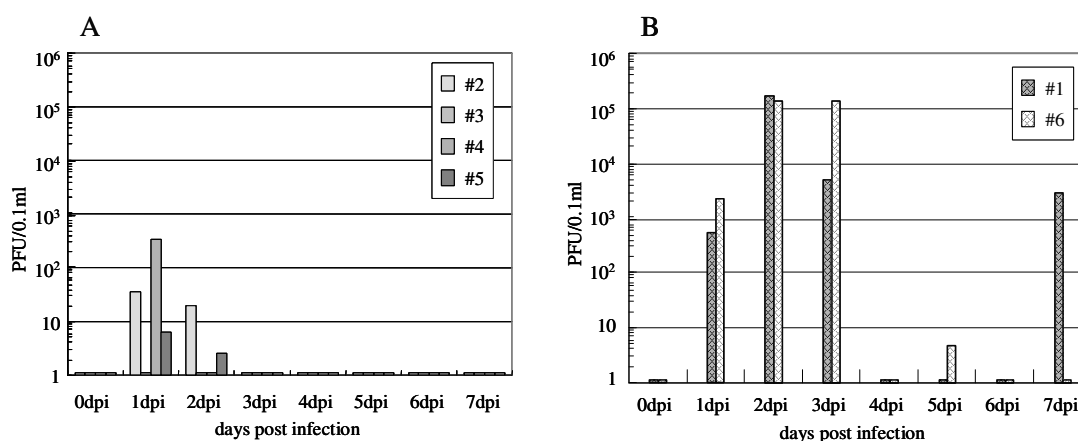


Figure 1: Viremia on pigs infected with FMDV strain O/TAW/97. A: T-1105 administered pigs (n=4), B: T-1105 non-administered pigs (n=2)

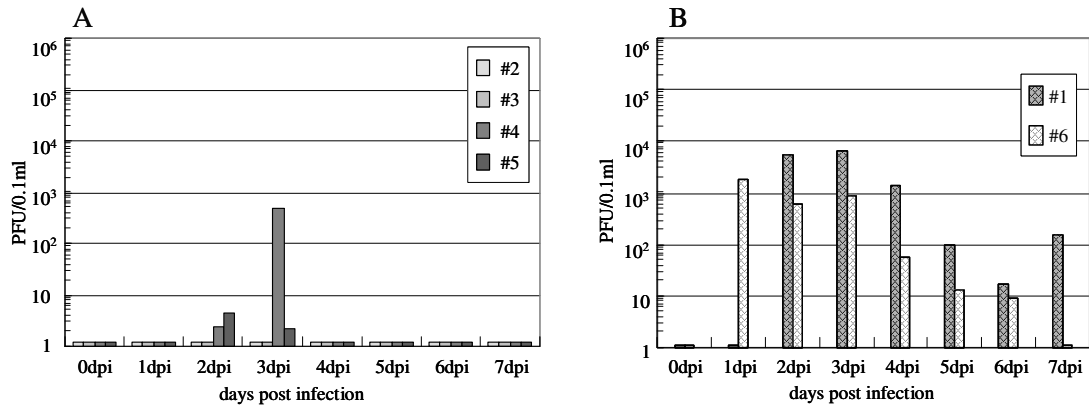


Figure 2: Virus excretion in saliva from pigs infected with O/TAW/97 A: T-1105 administered pigs (n=4), B: T-1105 non-administered pigs (n=2)

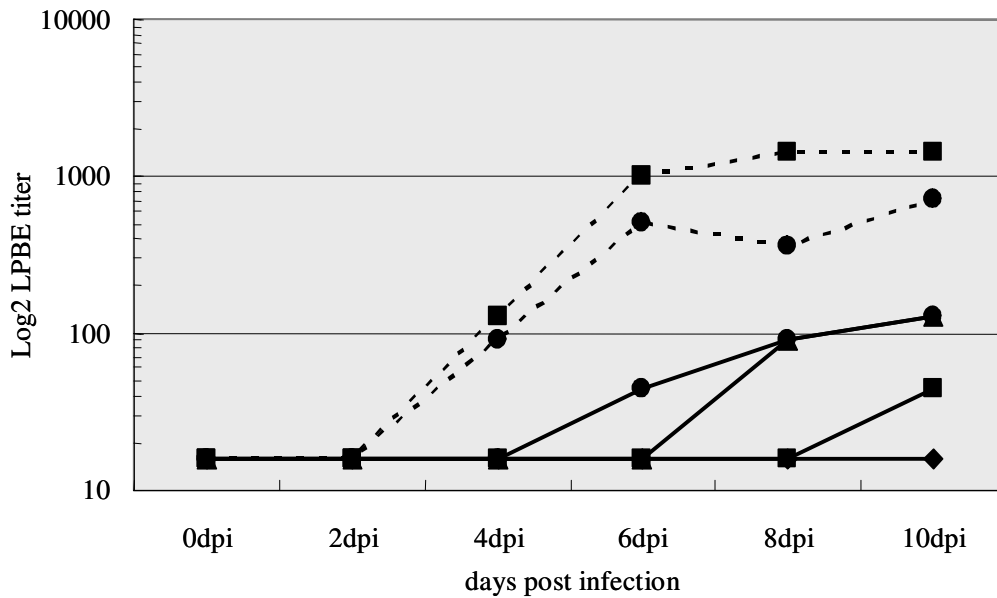


Figure 3: LPBE antibody responses. Solid lines and dotted lines indicate T-1105-administrated pigs (n=4) and control pigs (n=2), respectively.

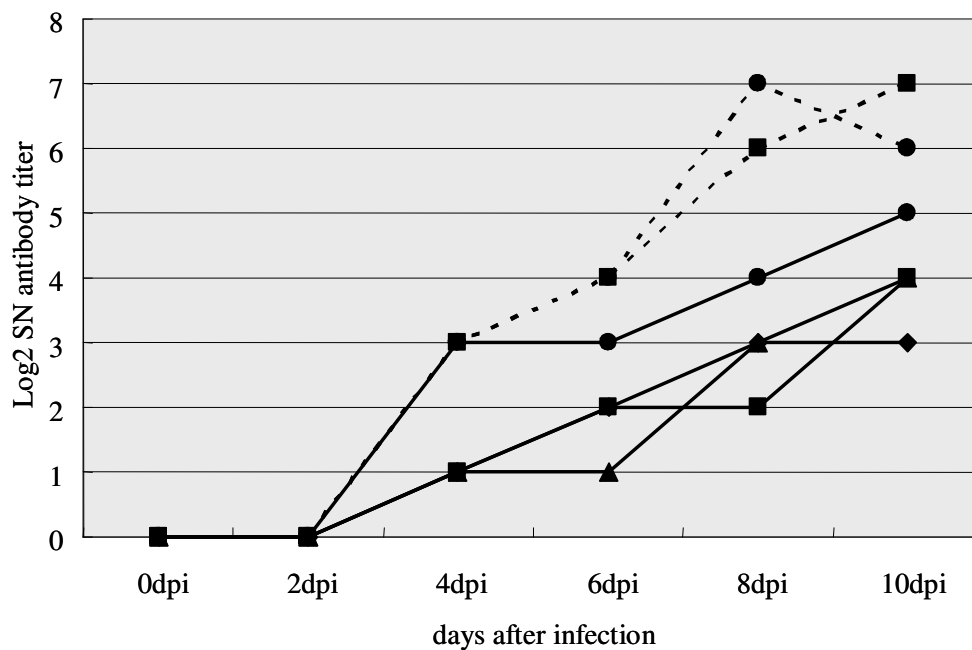


Figure 4: Serum neutralizing antibody responses. Solid lines and dotted lines indicate T-1105-administrated pigs (n=4) and control pigs (n=2), respectively.