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ORIGINAL ARTICLE

Sensitivity of FCV to recombinant feline interferon (rFeIFN)

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Abstract Feline calicivirus cause feline respiratory diseases, and inactivated and attenuated vaccines are available for its prevention. Moreover, the presence of vaccine breakdown strains (VBS) is problematic. In Japan, feline recombinant interferon (rFeIFN) has been used for its treatment. However the method of compare with each strains has not established. To examine the relationship between the breakdown vaccine strain and rFeIFN sensitivity, the sensitivity of 47 field isolates to rFeIFN was determined. The Log PDD_{50} values were normally distributed within the range 1.1–3.7, with a mean value of 2.3±0.64. Since 68.3% of the PDD values fell in the range of the mean ± standard deviation, the values in the range 1.7–2.9, the lower values, and the higher values were defined as representing moderate, low, and high sensitivity, respectively. Among the 15 vaccine breakdown strains, strain Fukuoka9 showed a low sensitivity, but strains ML89, T58, and N74 were highly sensitive, showing no association with vaccine breakdown. The amino acid sequence changes specific to the low rFeIFN-sensitive Fukuoka-9 strain were found, suggesting that these sites are involved in rFeIFN sensitivity.

Keywords rFeIFN · FCV · VBS

Introduction

Feline calicivirus infection (FCI) is a highly infectious upper airway disease, and is as important as feline viral rhinotracheitis caused by feline herpes virus among feline respiratory diseases (Gaskell and Dawson 1998). It has recently been reported that FCI accounts for a higher ratio of feline upper airway infections than feline viral rhinotracheitis

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in Japan (Mochizuki et al. 2000). Moreover, the presence of vaccine breakdown strains (VBS), which establishes infection in vaccinated cats, is problematic (Ohe et al. 2007).

Inactivated and attenuated vaccines are available for its prevention (Pedersen 1993). As for treatment, antibiotics are effective for the prevention of secondary infections (Pedersen 1993). Removal of viscous secretion, fluid replacement for dehydration, and symptomatic treatment with various vitamins to promote mucosal regeneration are also performed. In Japan, feline recombinant interferon (rFeIFN, Intercat, Toray) was marketed in 1994 as a therapeutic drug, and its efficacy was reported (Uchino et al. 1998).

IFN was reported as a virus replication-inhibitory factor by Nagano et al. in 1954 (Nagano and Kojima 1954), and named interferon by Issac et al. of England in 1957 (Isaacs and Lindenmann 1987). Its antiviral effect in cats has also been reported. Fulton et al. investigated the sensitivity of FCV (F9 strain) by plaque assay using natural feline IFN (NDV-FL) and 2 types of recombinant human IFN (HuIFN-αA and HuIFN-αA/D), and found that all IFN reduced the number of plaques, suggesting their efficacy (Fulton and Bunge 1985). Yanai et al. developed rFeIFN in 1990 (Yanai et al. 1990, 109th The Japanese Society of Veterinary Science), and Yamamoto et al. confirmed its antiviral action against feline immunodeficiency virus (FIV), feline infective peritonitis virus (FIPV), feline leukemia virus (FeLV), feline herpes virus (FHV), and FCV in vitro in 1991 (Yamamoto K. J. et al. 1990, 110th The Japanese Society of Veterinary Science). Subsequently, the therapeutic effect was observed in SPF cats experimentally infected with FCV (Ninomiya et al. 1991). Good outcomes were also obtained in clinical field studies (Uchino et al. 1991). The mean effective rate against FCI was 89.7%, and rFeIFN was ineffective in 2 of 19 animals in which FCV was detected (Uchino et al. 1992). In an rFeIFN distribution experiment performed by Ueda et al., rFeIFN was distributed at a high level in the region from the salivary gland over the pharyngolarynx 3 hours after intravenous administration (Ueda et al. 1993). rFeIFN was marketed as Intercat, and a 3-year field survey of its therapeutic effect on FCI was performed from 1994. The effective rate was 90%, but ineffectiveness was also noted although the rate was low (5/160) (Uchino et al. 1998). Genotype-associated variation in sensitivity is present in human viruses, such as HCV, but there has been no report on rFeIFN sensitivity of FCV field isolates.

Materials and methods

Viruses

Forty-four FCV strains isolated from cats brought to 4 animal hospitals in Japan between 1989 and 2001, F9 (ATCCVR782), CFI/68 (ATCCVR654), and FCV-255 strains and 11 strains newly isolated between 2001 and 2003 were used. As VBS, FCV-B, S1, H10, and Ao198-1 strains and 11 newly isolated strains, 15 strains in total, were used (Table 1).

rFeIFN sensitivity test

The rFeIFN sensitivity test established by Wagner (1961) was used. The plaque formation method (indirect agar layer method) confirmed to be quantitative by Lindenmann and Gifford (1963) was partially modified. For rFeIFN, Intercat (Kyoritsu Shoji, Japan) was dissolved with PBS(-) and adjusted to 10^7 units (U/ml), and monolayer CRFK cell cultures were sensitized with 1 ml of dilutions (0,10^2, 10^3, 10^4, and 10^5 U/ml) at 37°C. After 24 hours, the rFeIFN solution was removed, and the cells were inoculated with 30–

Table 1 Information on FCV isolates used for rFeIFN sensitivity test in this study

Strains	GenBank	Genogroups	Clusters	Log_{10} PDD_{50}	rFeIFN sensitivity
H17	AB100689	II	IIa	1.1	low
FCV-43	AB055452	I	C2	1.2	
FCV-2	AB055446	II	IIa	1.3	
ML19	AB100693	I	C1	1.3	
S2	AB055462	II	IIb	1.4	
IJ-F-3	AB058660	I	C6	1.5	
K9	AB058656	II	IIa	1.5	
ML1	AB055454	II	IIa	1.5	
ML3	AB055456	I	C2	1.5	
K4	AB058652	II	IIa	1.7	moderate
ML2	AB055455	II	IIb	1.7	
FCV-11	AB055449	II	IIa	1.9	
IJ-F-7	AB058663	I	C3	1.9	
K2	AB058650	I	C1	1.9	
ML6	AB055459	II	IIb	1.9	
IJ-F-4	AB058661	II	IIa	2	
ML5	AB055458	II	IIb	2	
K10	AB058657	II	IIa	2.1	
ML12	AB100690	I	C1	2.1	
ML13	AB100691	I	C5	2.1	
ML7	AB055460	I	C3	2.1	
IJ-F-9	AB058664	II	IIa	2.2	
K1	AB058649	I	C3	2.3	
K12	AB058659	I	C5	2.3	
FCV-170	AB055453	II	IIb	2.5	
IJ-F-5	AB058662	II	IIa	2.5	
K5	AB058653	I	C6	2.5	
ML4	AB055457	I	C2	2.5	
TOKYO 1	AB100694	II	IIb	2.5	
IBARAKI 1	AB055445	I	C6	2.6	
FCV-1	AB100687	II	IIa	2.7	

Table 1 (continued)

Strains	GenBank	Genogroups	Clusters	Log_{10} PDD_{50}	rFeIFN sensitivity			
FCV-6	AB055448	II	IIa	2.7				
FCV-B (VBS)	AB055451	II	IIb	2.7				
S21	AB055463	I	C2	2.7				
FCV-255	U06646	I	C5	2.8				
K8	AB058655	I	C6	2.8				
S1 (VBS)	AB055461	II	IIb	2.9				
Ao198-1 (VBS)	AB190197	II	IIa	2.9				
FCV-5	AB055447	I	C7	2.9				
H10 (VBS)	AB058648	I	C1	2.9				
S103	AB058665	I	C7	2.9				
F9	M86379	I	C7	2.9				
K11	AB058658	I	C7	3	high			
CFI/68	U13992	II	C3	3.3				
K7	AB058654	I	IIa	3.3				
ML14	AB100692	I	C7	3.4				
K3	AB058651	I	C7	3.7				
mean±SD				2.3 ± 0.64				
VBSs	Year	Country	Location	Disease Complex	GenBank	Genogroups	Log_{10} PDD_{50}	rFeIFN sensitivity
Fukuoka9	2001	Japan	Fukuoka	Mild upper respiratory	–	II	1.2	low
S175	2001	Japan	Kanagawa	Mild upper respiratory	–	I	2.7	moderate
S280	2001	Japan	Kanagawa	Mild upper respiratory	–	I	1.8	moderate
S292	2001	Japan	Kanagawa	Mild upper respiratory	–	I	2.7	moderate
ML89	2002	Japan	Osaka	Mild upper respiratory	–	II	3.4	high
IK30	2003	Japan	Kagoshima	Acute respiratory	–	I	1.7	moderate
T34	2003	Japan	Tokushima	Upper respiratory	–	II	2.9	moderate
T58	2003	Japan	Tokushima	Upper respiratory	–	I	3.1	high
N45	2003	Japan	Nagano	Mild upper respiratory	–	I	2.9	moderate
N74	2003	Japan	Nagano	Upper respiratory	–	II	3.1	high
N81	2003	Japan	Nagano	Upper respiratory	–	I	1.9	moderate

100 PFU/dish viral suspension. After adsorption at 37°C for 2 hours, the viral suspension was removed, and plaque agar was layered. Plaque agar was prepared by mixing 1.8% sea plaque agar (Cambrex Bio Science Rockland Inc.) with an equivalent volume of MEM at a 2-times higher concentration, 10% calf serum, and 1% neutral red (Wako Pure Chemicals). After incubation at 37°C for 72 hours, the cells were stained with Giemsa's solution (Merck Ltd., Japan), the diameter and number of plaques were measured, and the 50% plaque-depressing dose (PDD_{50}) was calculated.

Representing the number of plaques of samples at each IFN dilution factor as p, that of the control as P, the plaque diameter of sample s as d, and that of the control as P, the ratio of viral replication ability was presented as $p/P \times (d/D)^2$ (Lindenmann and Gifford 1963; Wagner 1961). An approximate line was drawn by plotting this value on the Y-axis and the exponent of the reciprocal of the IFN dilution factor on the X-axis. Substituting Y of this approximate line for 0.5 (50%), the exponent of PDD_{50} was obtained by calculating X. The correlation coefficient was simultaneously calculated to assess the reliability. The test was repeated twice or more for each isolate, and the mean was regarded as PDD_{50}.

Genogrouping

As a genogrouping method, preparation of a phylogenetic tree by the NJ method using the genes in the capsid protein-coding region has been reported. Isolates with a 90% or higher bootstrap value on 1000-time repeats were regarded as a genogroup, and those grouped under a genogroup were regarded as a cluster. The genogroups were determined by the method reported by Sato et al. (2002a) using software of Clustal X (Sato et al. 2002b).

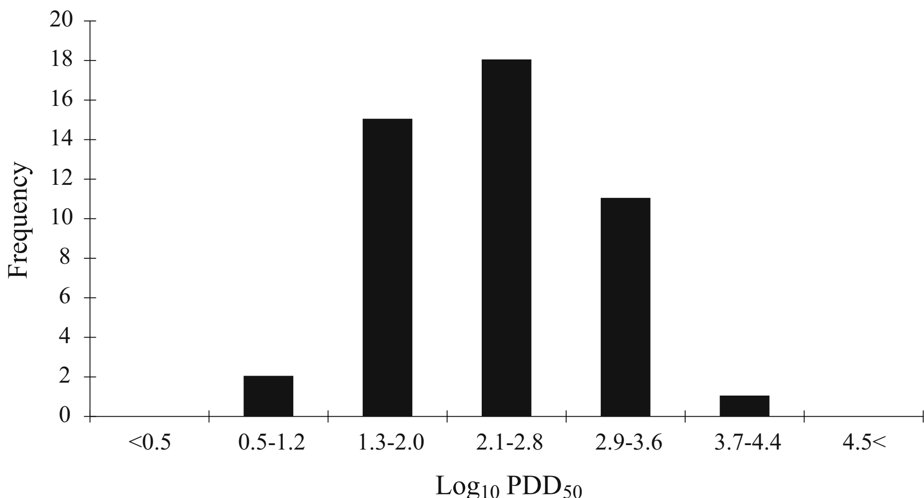

Fig. 1 Histogram of PDD_{50} of the 47 strains. PDD_{50} of the 47 strains tested showed normal distribution PDD_{50} of the 47 strains tested showed 68.3% of the strains whose PDD_{50} fell within the range of population 2.3±0.65 (mean±S.D) were determined to have moderate to have moderate sensitivity. The strains with PDD_{50} below and above these values were considered to have low and high sensitivity

Table 2 Comparative analysis of the capsid genogroups and rFeIFN sensitivity of each strain

Genogroups	rFeIFN sensitivity			Total
	low	moderate	high	
I	4/25 (16%)	17/25 (68%)	4/25 (16%)	25
II	5/22 (23%)	16/22 (72%)	1/22 (5%)	22
Total	9	33	5	47

Results

rFeIFN sensitivity test

The PDD_{50} values of the 47 strains showed a normal distribution ranging from $10^{1.1}$ to $10^{3.7}$ (Fig. 1). The mean and standard deviation were $10^{2.3}$ and $10^{0.64}$, respectively, and 68.3% of the strains were included within the range of the mean ± standard deviation. Strains with values within this range were judged moderately sensitive, and strains with lower and higher values were judged low- and high-sensitive, respectively. Table 1 lists the strains in the order of rise in PDD_{50} (Table 1).

rFeIFN sensitivity and genogroups

Molecular phylogenetic analysis of nucleotide sequences of the 47 strains and 20 strains registered in Genebank was performed. The strains were divided into Genogroups I and II (GI and GII), and GII was further divided into Clusters IIa and IIb. GI was divided into 7 clusters (C1–C7) (Table 1).

The genogroups of the strains and comparison of rFeIFN sensitivity are shown in Table 2. In GI, 16% (4/25), 68% (17/25), and 16% (4/25) of strains were low-, moderately, and high-sensitive, respectively. In GII, 23% (5/22), 72% (16/22), and 5% (1/22) of strains were low-, moderately, and high-sensitive, respectively (Table 2). On comparison of rFeIFN sensitivity and clusters (C1–C7, IIa, and IIb), (Table 3).

rFeIFN sensitivity of vaccine breakdown strains

Of the 15 vaccine breakdown strains, only Fukuoka 9 was low-sensitive, and ML89, T58, and N74 were high-sensitive (Table 1).

Table 3 Relationship between rFeIFN sensitivity and clusters

Genogroups	Clusters	rFeIFN sensitivity			Total
		low	moderate	high	
I	C1	1	3	0	4
	C2	2	3	0	5
	C3	0	3	1	4
	C5	0	3	0	3
	C6	1	3	0	4
	C7	0	2	3	5
II	IIa	4	9	1	14
	IIb	1	7	0	8
Total		9	33	5	47

Discussion

To investigate the association of VBS with rFeIFN sensitivity, we measured the rFeIFN sensitivity of the field isolates. Using a method that takes the rFeIFN dilution factor and the number and diameter of plaques into account, the rFeIFN concentration and PDD_{50} value were inversely proportional in all strains. The diameter decreased in some strains, but remained unchanged in others. Reduction of the number of plaques represents the direct effect of IFN on viruses, and the reduction of the plaque diameter represents a decrease in viral replication ability. Evaluations based on the number of plaques alone and using $TCID_{50}$ as an index reflect only one of these. It was suggesting that this method is better for sensitivity comparison of many field strains than methods using the number of plaques alone (Fulton and Bunge 1985) and $TCID_{50}$ (Mochizuki et al. 1994).

Uchino et al. (1998) reported that the effective rate of rFeIFN (Intercat) was 90% (markedly effective: 13%, effective: 77%), and rFeIFN was not very effective for the remaining 10% (slightly effective: 6%, ineffective: 3%, aggravated: 1%) (Uchino et al. 1998). In our study, 9/47 (19%) were low-sensitive, which was slightly higher than the ineffective rate reported by Uchino et al. However, viruses were not isolated in their study, and the effective rate was determined based on only signs. Thus, the number of virus-excreting strains may have been higher than the ineffective rate. Mochizuki et al. reported that the antiviral effect of rFeIFN was dependent on the dose in vivo, and the sensitivity was dependent on the virus species and host cell system (Mochizuki et al. 1994), but there has been no report on the sensitivity of field viruses. In actual treatment, a healing mechanism involving immune function may act in infected animals, in addition to the action of IFN. No complete absence of the sensitivity was noted in any field strain tested.

Regarding the relationship between the rFeIFN sensitivity and genogrouping, more low-sensitive strains were included in GII than in GI. Human hepatitis C virus (HCV) was divided into 4 genogroups, and IFN is more effective for Genotypes III and IV, whereas IFN is ineffective for many strains in Genotype II (Orito et al. 1994). In FCV, moderately and high-sensitive strains accounted for 77%, showing that the effect does not markedly vary among the genogroups, unlike HCV.

Of the 15 vaccine breakdown strains, only Fukuoka 9 was low-sensitive, and ML89, T58, and N74 were high-sensitive, showing no association between the vaccine breakdown and low rFeIFN sensitivity. However, GII consisted of only Japanese strains (Sato et al. 2002b), and included many strains with low rFeIFN sensitivity. Attention should be paid to the trend of such low-sensitive strains. Since this study showed that the effect of rFeIFN varied, clinicians should be more careful in the treatment of FCV infection. Fluid replacement to maintain normal body fluid level and immunopotentiation may represent other measures.

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