Identification of *Inonotus obliquus* polysaccharide with broad-spectrum antiviral activity against multi-feline viruses

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ABSTRACT

Inonotus obliquus polysaccharides (IOPs) are a potential drug for the prevention and treatment of cancer, cardiopathy, diabetes, AIDs, pancreatitis and other diseases. In this study, we found that IOP can act as a broad-spectrum antiviral drug against feline viruses in the *in vitro* experiment. Using cell models of feline calicivirus (FCV), we demonstrated that IOP treatment was capable of exhibiting anti-FCV strain F9 activity in cell-based assays and also showed low cytotoxicity. Investigation of the mechanism of action of the compound revealed that IOP treatment induces its inhibitory actions directly on virus particles through blocking viral binding/absorpting. The inhibitory activity against other FCV isolates from China was also identified. More importantly, we found that IOP exhibited broad-spectrum antiviral activity against the feline herpesvirus 1, feline influenza virus H3N2 and H5N6, feline panleukopenia virus and feline infectious peritonitis virus that can contribute to respiratory and gastrointestinal diseases in cats. These findings suggest that IOP may be a potential broad-spectrum antiviral drug against feline viruses.

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1. Introduction

Respiratory and gastrointestinal viruses, such as feline calicivirus (FCV), feline herpesvirus 1 (FHV-1), feline influenza virus (FIV), feline panleukopenia virus (FPV) and feline infectious peritonitis virus (FIPV) are the main causes that contribute to acute and chronic infections in cats. Currently, while widespread vaccination projects have been performed, the prevalence of these viruses remains high due to main antigen change and the emergence of new viruses. Immune serum containing viral antibodies can be used to prevent infection in susceptible animals. However, the method is not currently widely applied. Feline recombinant interferon-omega is effective in the treatment of feline leukemia virus (FeLV) [1] and FCoV [2] infection in cats and also inhibits viral replication of canine parvovirus [3]. It remains unclear if it is an effective treatment to inhibit other feline viral infections. Mefloquine, a small molecule inhibitor was demonstrated as an effective antiviral drug against FCoV [4] and FCV [5] replication in *in vitro* experiments. Our previous studies showed that LiCl [6] and germacrone [7] can efficiently inhibit FCV replication *in vitro*. However,

the broad-spectrum antiviral activity of the inhibitors needs to be investigated further. So, it is urgent to develop an effective, safe and broad-spectrum antiviral drug for feline viral diseases.

Inonotus obliquus (IO), also known as Chaga, is a white-rot fungus belonging to the Hymenochaetaxeae family of Basidiomycotina [8]. It is a parasite on Betula (birch) trunks in low latitudes (approximately 45–50°N) of Asia, Europe, and North America [8]. Clinical animal experiments demonstrate that IO polysaccharides may be a potential drug to prevent and cure cancer, cardiopathy, diabetes, AIDs, pancreatitis and other diseases [9–11]. IO fungus water extract shows a virucidal activity towards hepatitis C virus [12], suggesting a potential application in antiviral research. However, it is a crude extract, and the main antiviral factor remains unknown. Moreover, a crude extract may potentially exhibit stronger toxicity. Here, we evaluate the antiviral effects of IO polysaccharides (IOPs) towards multi-feline respiratory and gastrointestinal viruses such as feline calicivirus (FCV), feline herpesvirus 1 (FHV-1), feline influenza virus (FIV), feline panleukopenia virus (FPV), feline infectious peritonitis virus (FIPV). The results revealed that IOP possesses broad-spectrum activity against feline RNA- and DNA-viruses. Preliminary mechanism revealed that IOP acts by targeting virus entry.

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2. Materials and methods

2.1. Compounds

The IOP was a gift from Professor Juanjuan Qu of the College of Resources and Environmental Science of Northeast Agricultural University. Preparation of the IOP has been described previously [8]. It was initially dissolved in DMEM at a concentration of 50 mg/mL. Ribavin was purchased from Sigma-Aldrich (Sigma, St. Louis, MO, USA).

2.2. Cell lines and viruses

Crandell-Reese feline kidney (CRFK) cells and Madin-Darby Canine Kidney (MDCK) cells (ATCC) were grown in Dulbecco's modified Eagle medium (DMEM, Gibco) containing 8% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The cells were incubated at $37\,^\circ$C in a 5% CO_2 humidified cabinet. FCV, FHV-1, FPV and FIPV were propagated and titrated in CRFK cells. A/feline/Heilongjiang/LCG/2015 (H3N2) was propagated in 10-day-old embryonic special-pathogenic-free (SPF) chicken eggs and titrated in CRFK cells. A/feline/Heilongjiang/LS/2015 (H5N6) was propagated and titrated in MDCK cells. The recombinant FCV strain 2280 expressing the eGFP (rFCV-eGFP) was produced by our lab, and the eGFP was inserted into the N terminus of VP1. The recombinant FHV strain VR-814 expressing the eGFP (rFHV-eGFP) was produced using a homologous recombination method, and the US3 gene was replaced by the CMV-eGFP expression cassette. Both recombinant viruses were produced by our lab.

2.3. Cell viability assay

Cells were inoculated in a 96-well plate (10^4 cells/well) prior to assay. The next day, IOP or Ribavin at different concentrations diluted in DMEM containing 1% FBS, was added to the cells. Cells treated with only DMEM containing 1% FBS were used as a control. Following treatment for 24 or 72 h, cytotoxicity assays were performed using a Cell Counting Kit-8 (CCK8) (Donjindo, Japan). After washing three times with $1 \times$ PBS, a CCK8 solution ($20\,\mu$L) and DMEM ($80\,\mu$L) were added to the cells, and the plate was incubated at $37\,^\circ$C for two hours. The optical density (OD) was determined by an EnSpire® Multimode Plate Reader (PE, USA) under a 450 nm excitation filter [13]. The relative cell viability was calculated as a percentage of that of the control cells.

2.4. Antiviral examination using CPE inhibition and viral growth assays

Cells were inoculated in 96-well plate (10^4 cells/well) prior to assay. The next day, IOP or ribavin at different concentrations was added to the cells. Following 2 h of treatment, viruses diluted in DMEM in the presence of drug were added to cells for 1 h. After the cells were washed three times, the cells were inoculated with medium containing IOP for 24–72 h. When CPE with 100% was observed in the control cells, the supernatants were harvested for viral titration, the cells were washed three times, and medium containing $100\,\mu$g/mL of resazurin ($100\,\mu$L/well) was added. After 3 h of incubation, fluorescent signals were measured with an EnSpire® Multimode Plate Reader (PE, USA) using a 544 nm excitation filter and 590 nm emission filter. The percentage inhibition of CPE was calculated by:

$$\text{CPE inhibition}(\%) = \frac{A - B}{C - D} \times 100$$

where A is the fluorescence intensity of infected cells treated with IOP; B is the fluorescence intensity in infected cells untreated with IOP; and C is the fluorescence intensity of uninfected cells untreated with IOP.

2.5. The effect of IOP on the growth of reporter viruses expressing eGFP

The preparation of cells and the application strategy of drug during infection were as described in the CPE inhibition assay. The cells were infected with rFCV-eGFP or rFHV-eGFP, and the fluorescence intensity assay was performed at 24 h post-infection (hpi).

2.6. Time of addition assay

To determine which stages of the viral life cycle were inhibited by IOP, the antiviral activity in pre-treatment, simultaneous treatment and post-treatment manner were investigated. Cells seeded in a 48-well plate (10^5 cells/well) were cultured for 24 h. (1) The cells were pre-treated with IOP at a concentration of $60\,\mu$g/mL for two hours. After incubation, the cells were inoculated with strain F9 at an MOI of 0.01. After one hour of absorption, the medium was replaced with fresh DMEM containing 1% FBS and $60\,\mu$g/mL of IOP. (2) The cells were simultaneously exposed to strain F9 at an MOI of 0.01 and IOP ($60\,\mu$g/mL). After one hour of absorption, the medium was replaced with fresh DMEM containing 1% FBS and $60\,\mu$g/mL of IOP. (3) The cells were inoculated with strain F9 at an MOI of 0.01 for 1 h prior to exposure to $60\,\mu$g/mL IOP at 1, 2, 4 and 6 hpi. The recovered virus yields in cell supernatants were determined at 36 hpi. The levels of vRNA were examined by real-time RT-PCR according the method described previously [6].

2.7. Viral titer tests

The protocol for virus titration was described in our previous study [14]. Briefly, ten-fold diluted virus stocks were prepared, and cells were inoculated with 0.1 mL of each dilution. After 1 h of absorption, the supernatant was discarded, and fresh DMEM containing 1% FBS and 1% penicillin-streptomycin was added to each well. The cytopathology at 72 hpi was observed, and the viral titers were expressed as the median tissue culture infective dose $\log_{10}$ ($TCID_{50}$/ml) according to the method of Reed and Müench [15].

For FPV infection experiment, the viral replication was determined *via* direct fluorescence assay (DFA) using FITC-conjugated anti-Canine Parvovirus monoclonal antibody (CJ-F-CPV-MAB, VMRD).

2.8. Statistics

The data are presented as the means $\pm$ standard deviation (SD). Statistical significance was determined using unpaired *t* tests and a one-way ANOVA in Prism 5.0 software (GraphPad Software). For all tests, a value of $p < 0.05$ was considered to indicate a significant difference.

3. Results

3.1. IOP inhibits FCV strain F9 replication in vitro

A recombinant FCV expressing green fluorescent protein (rFCV-eGFP) as a reporter for virus replication was used to analyze IOP for inhibitor of FCV replication. As shown in Fig. 1A, we found that IOP treatment reduced the fluorescence intensity, which depended on the dose of IOP. It revealed that IOP may act as a potent inhibitor of FCV. Next, the cytotoxicity of IOP was evaluated in CRFK cells using a CCK8 assay. Its 50% cytotoxic concentration (CC_{50}) was determined to be greater than 1 mg/mL during 24 and 72 h incubation periods (Fig. 1B), suggesting that the treatment exhibited low cytotoxicity.
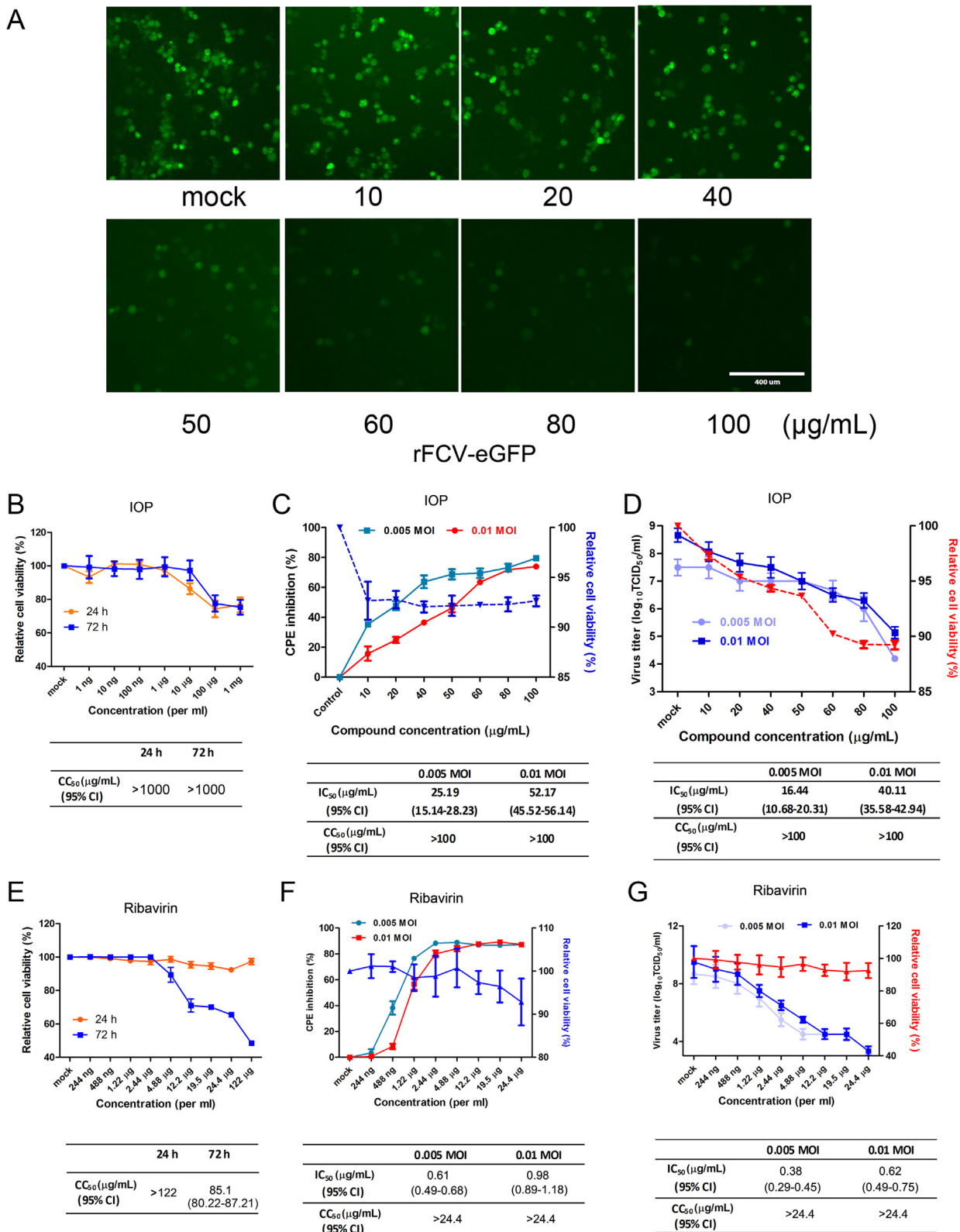
Fig. 1. Evaluation of the antiviral activity of IOP against FCV strain F9.
(A) CRFK cells pretreated with different concentrations of IOP for 2 h were infected with rFCV-eGFP at an MOI of 0.01 in the presence of IOP. At 24 hpi, the fluorescence was observed. (B, E) CRFK cells were treated with different concentrations of IOP (B) or ribavirin (E) diluted in DMEM containing 1% FBS, and the cell viability was determined after a 24 h or 72 h incubation periods using a CCK8 assay. (C, F) CRFK cells (10^4) pretreated with different concentrations of IOP (C) or ribavirin (F) for 2 h were infected with strain F9 at various MOIs in the presence of IOP. A CPE inhibition assay was performed using the resazurin-based method at 24 hpi. Cell viability was determined using a CCK8 assay. (D, G) According to the method described in C and F, the virus yield reduction in the supernatants of IOP-treated cells (D) or ribavirin-treated cells (G) and the cell viability at 24 hpi were determined. Results represent the mean of three replicates ± SD, and each experiment was repeated three times.

To further evaluate the anti-FCV activity, the resazurin-based CPE inhibition assay as described previously [5] was used. Titration of IOP showed a concentration–response relationship, showing nearly 80% inhibition of the cytopathic effect (CPE) at a concentration of 100 μg/mL and 30% inhibition at a concentration of 10 μg/mL when the cells were infected at an MOI of 0.005 (Fig. 1C). As the number of viruses exposed to cells increases, the ability to inhibit the formation of CPE was decreased and the 50% inhibitory concentration (IC_{50}) was increased from 25.19 μg/mL at an MOI of 0.005 to 52.17 μg/mL at an MOI of 0.01. Moreover, a significant therapeutic index (TI) (CC_{50}/IC_{50}) value for IOP ranging from >1.9 to >4.0 was determined. CPE inhibition was associated with significant reductions in the progeny virion loads. A concentration-dependent inhibition of viral replication was also demonstrated in assays from an MOI of 0.005 to an MOI of 0.01 (Fig. 1D). Compared with the control cells, viral titers from IOP (100 μg/mL) treated cells were reduced by 3.2 log at an MOI of 0.005 and by 1.3 log at an MOI of 0.01. The calculated IC_{50} are shown in Fig. 1D.

In this experiment, ribavirin, a known polymerase inhibitor of RNA viruses, was included as a positive control. Cytotoxicity test for ribavirin in CRFK cells showed that treatment with it exhibited low toxity at 24 h post-inoculation, but exhibited significant toxity at 72 h post-inoculation (Fig. 1E) and CC_{50} was determined as 85.1 μg (Fig. 1E). In the resazurin-based CPE inhibition assay, IC_{50} was determined as 0.61 μg/mL at an MOI of 0.005 and 0.98 μg/mL at an MOI of 0.01 (Fig. 1F). In the virus yield reduction assay, IC_{50} was determined as 0.38 μg/mL at an MOI of 0.005 and 0.62 μg/mL at an MOI of 0.01 (Fig. 1F). While the antiviral activity of ribavirin was more potent that IOP, the in vitro toxicity testing revealed that ribavirin exhibited higher toxicity than IOP.

3.2. IOP treatment directly blocks viral infectivity

To determine which steps of viral replication are disrupted, antiviral activities in the pre-treatment (−2 h), simultaneous treatment (0 h) and post-treatment (+1 h to +6 h) were investigated. At 24 hpi, viral RNA levels and viral yields were determined. IOP treatment significantly reduced viral RNA levels at any time point (Fig. 2A) when cells were exposed to IOP. As the time of exposure to IOP was delayed, the inhibitory ability was decreased. When the cells were exposed to IOP at 6 hpi, a reduction of only 43.6% was detected (Fig. 2A). The results of the viral titer assay also showed a similar trend (Fig. 2B). IOP addition from −2 h to +4 h significantly inhibited viral replication (Fig. 2B). While viral RNA levels were reduced when cells were exposed to IOP at +6 h, the viral titration test showed that the viral production was not significantly decreased (Fig. 2B).

To further investigate the mechanism of the anti-FCV activity of IOP, anti-FCV activities during virus pre-treatment, cell pre-treatment and virus-cell simultaneous inoculation were compared. To investigate whether the anti-FCV activity of IOP resulted from the activation of cellular factors, cells were pre-treated with IOP; then cells in CP-A treated group were infected with FCV in presence of IOP, and cells in CP-B treated group were infected with FCV without IOP. As shown in Fig. 2C and D, the viral RNA level and viral titer from the CP-A treated group were significantly lower than the mock group but both levels were not reduced in the CP-B treated group while cells were pre-treated with IOP, indicating that anti-FCV activity for IOP may be not a result of activation of host factors by IOP treatment. Next, we examined whether IOP treatment induces its inhibitory actions directly on virus particles. The mixture containing virus and IOP was incubated at 4 °C for one hour and then added to cells. For the VT-A treated group, after a one-hour absorption period, the cells were continuously kept in the presence of IOP, but the VT-B treated group did not receive IOP in the medium. Both the viral RNA levels and the viral titers

from the VT-A and VT-B treated groups were significantly lower than the mock group, but no significant difference was observed between the VT-A and VT-B treated groups (Fig. 2C, D). In both the VS-A and VS-B group, the mixture containing virus and IOP was directly added to cells. For the VS-A treated group, after a one-hour absorption period, cells were continuously kept in the presence of IOP, but the VS-B treated group did not receive IOP in the medium. The result from a comparison between the VS-A and −B treated groups was similar to that between the VT-A and VT-B treated group, both of which indicate that IOP treatment may affect viral binding/absorpting to cells directly.

3.3. Antiviral efficacy against field isolates

IOP displays high antiviral activity against the reference strain FCV F9. Further, we analyzed the antiviral activity against other field FCV isolates. CPE-inhibition and virus yield reduction assays were performed. As shown in Fig. 3A, a nearly 50–60% inhibition of CPE induced by these field isolates was observed at a concentration of 60 μg/mL. As shown in Fig. 3B, IOP treatment led to a 2–2.5 log reduction in the production of these field isolate viruses.

3.4. IOP displays broad-spectrum antiviral activity

To investigate whether IOP displays broad-spectrum antiviral activity, we examined the replication of other viruses widely circulating in cat populations in the presence of IOP (Table 1).

IOP treatment led to the inhibition of CPE induced by FHV, which exhibited a concentration-response effect (Fig. 4A). The IC_{50} value was determined to be 18.15 μg/mL (Table 2). In the presence of IOP at 20 μg/mL or 60 μg/mL, viral titers were decreased by 1 or 1.6 log (Fig. 4B). To visualize the inhibitory effect of IOP against FHV replication, a recombinant FHV expressing the eGFP was used. As shown in Fig. S1 in the Supplementary material, we found that IOP treatment reduced the fluorescence intensity, which depended on the dose of IOP.

Moreover, IOP treatment also suppressed the CPE induced by FCoV infection (Fig. 4C), FPV infection (Fig. 4E), FIV H5N6 (Fig. 4G), and H3N2 (Fig. 4I) subtype infections; compared with each control, exposure to 60 μg/mL of IOP led to 2.5 log (Fig. 4D), 1.1 log (Fig. 4F), 1.6 log (Fig. 4H) and 1.0 log (Fig. 4J) reductions in the virus titers, respectively.

4. Discussion

Currently, rivavirin and nucleotide analogues as a nonspecific inhibitor of DNA/RNA synthesis exhibit a broad-spectrum antiviral activity, but application of these antiviral compounds, such as idoxuridine (5-iodo-2′-deoxyuridine) and trifluorothymidine (5-trifluoromethyl-2′-deoxyuridine) may affect the normal biological process required by host or is too toxic to be administered systemically [16]. Antiviral activity of several sulfated polysaccharides, such as heparin, dextran sulfate, pentosan polysulfate, mannan sulfate, and sulfated cyclodextrin, have been demonstrated in various enveloped viruses, including herpes simplex virus (HSV) [17,18], human cytomegalovirus [19], human immunodeficiency virus (HIV) [20], simian immunodeficiency virus [19], and flaviviruses [21]. The extracts of Ulva fasciata can inhibit the infection of Japanese encephalitis virus (JEV) [22].

Here, we describe the properties of a biological macromolecular, Inonotus obliquus polysaccharides (IOPs) that inhibits a broad spectrum of feline viruses currently circulating widely in cats. In this study, we found that IOP potently inhibits at least five different families including RNA viruses (Caliciviridae, Coronaviridae and Orthomyxoviridae) and DNA viruses (Alphaherpesvirinae and Parvovirus) (Table 1). To visualize the inhibitory effect of IOP, a
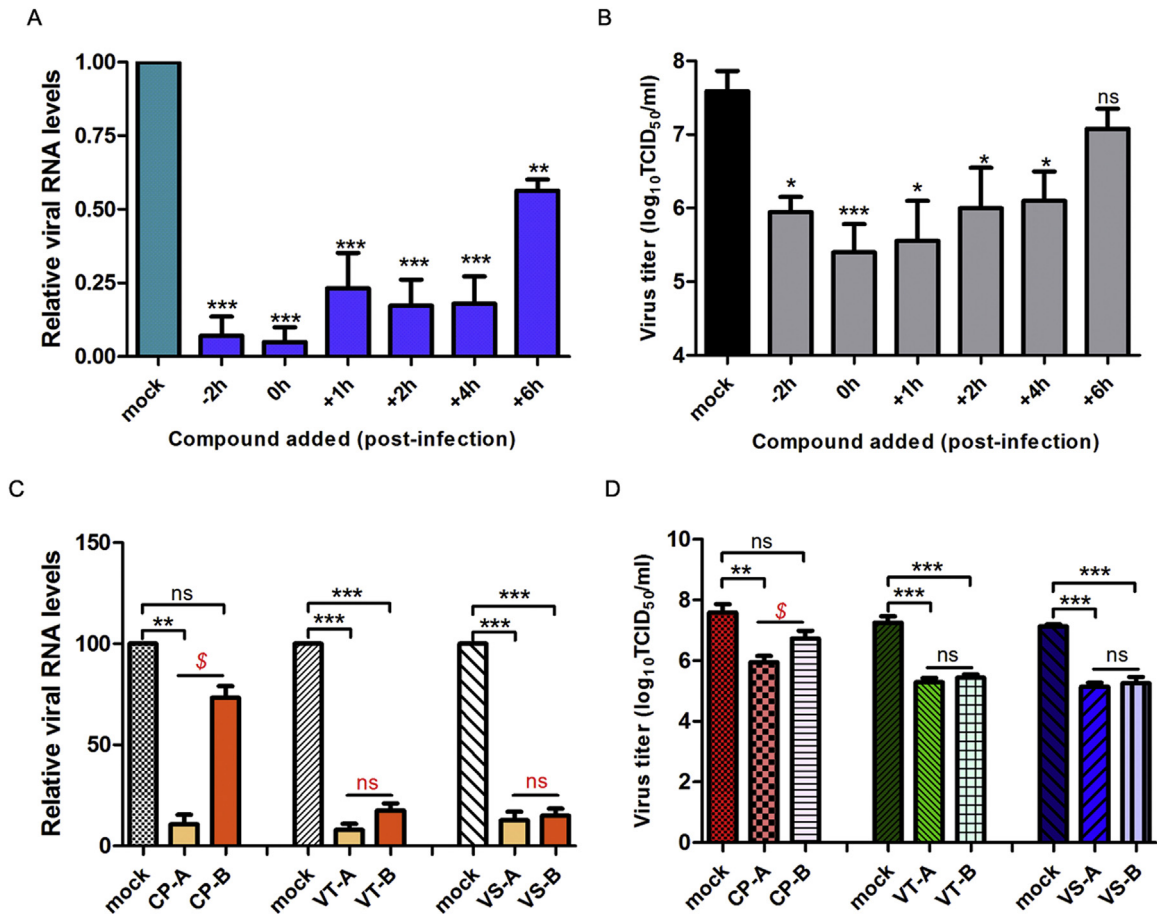
Fig. 2. Analysis of the preliminary antiviral mechanism for IOP.

(A, B) CRFK cells (10^4) were infected with strain F9 at an MOI of 0.01. IOP (60 μg/mL) was added to the cells at −2 h, 0 h, 1 h, 2 h, 4 h and 6 h. Viral RNA (A) and titers (B) were analyzed at 24 hpi. (C, D) CRFK cells were pre-treated with 100 μL of IOP (60 μg/mL) or DMEM (mock) for 2 h before infection in the CP-A and CP-B groups. In the CP-A group, the medium was not changed, and strain F9 at an MOI of 0.01 was directly added. In the CP-B group, the cells were washed and virus at an MOI of 0.01 was added. After one-hour absorption at 37 °C, fresh DMEM containing 1% FBS was added into the CP-A and CP-B groups. For the VT-A and VT-B groups, 100 μL of IOP (60 μg/mL) or DMEM (mock) and 10 μL of strain F9 (an MOI of 0.01 when added into cells) were mixed and incubated at 4 °C for one hour before the mixture was added to the cells. After one-hour absorption at 37 °C, the cells were washed, and fresh DMEM containing 1% FBS and IOP (60 μg/mL) or containing only 1% FBS was added into VT-A and VT-B groups, respectively. For the VS-A and VS-B group, 100 μL of IOP (60 μg/mL) or DMEM (mock) and 10 μL of strain F9 (an MOI of 0.01 when added into cells) were mixed and then the mixture was directly added to the cells. After one-hour absorption at 37 °C, the cells were washed, and fresh DMEM containing 1% FBS and IOP (60 μg/mL) or containing only 1% FBS was added to the VS-A and VS-B groups, respectively. Viral RNA (C) and titers (D) were analyzed at 36 hpi. Results represent the mean of three replicates ± SD, and each experiment was repeated three times.
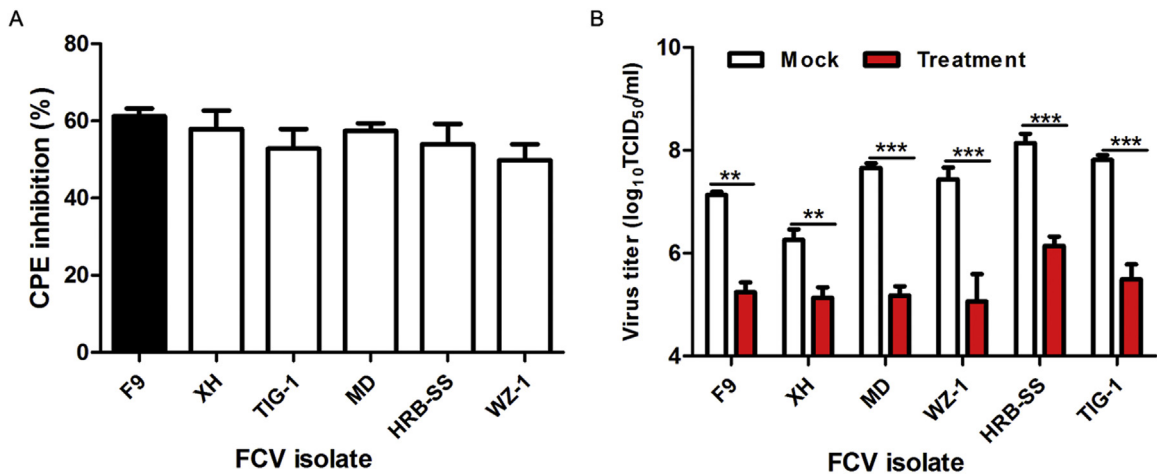

Fig. 3. Inhibition of multiple FCV field strains by IOP.

CRFK cells were infected at an MOI of 0.01 with the FCV strains. Infections were performed in presence of DMEM (mock) or IOP (60 μg/mL). The resazurin-based-CPE inhibition assay and viral titers were determined at 24 hpi. Results represent the mean of three replicates ± SD, and each experiment was repeated three times.
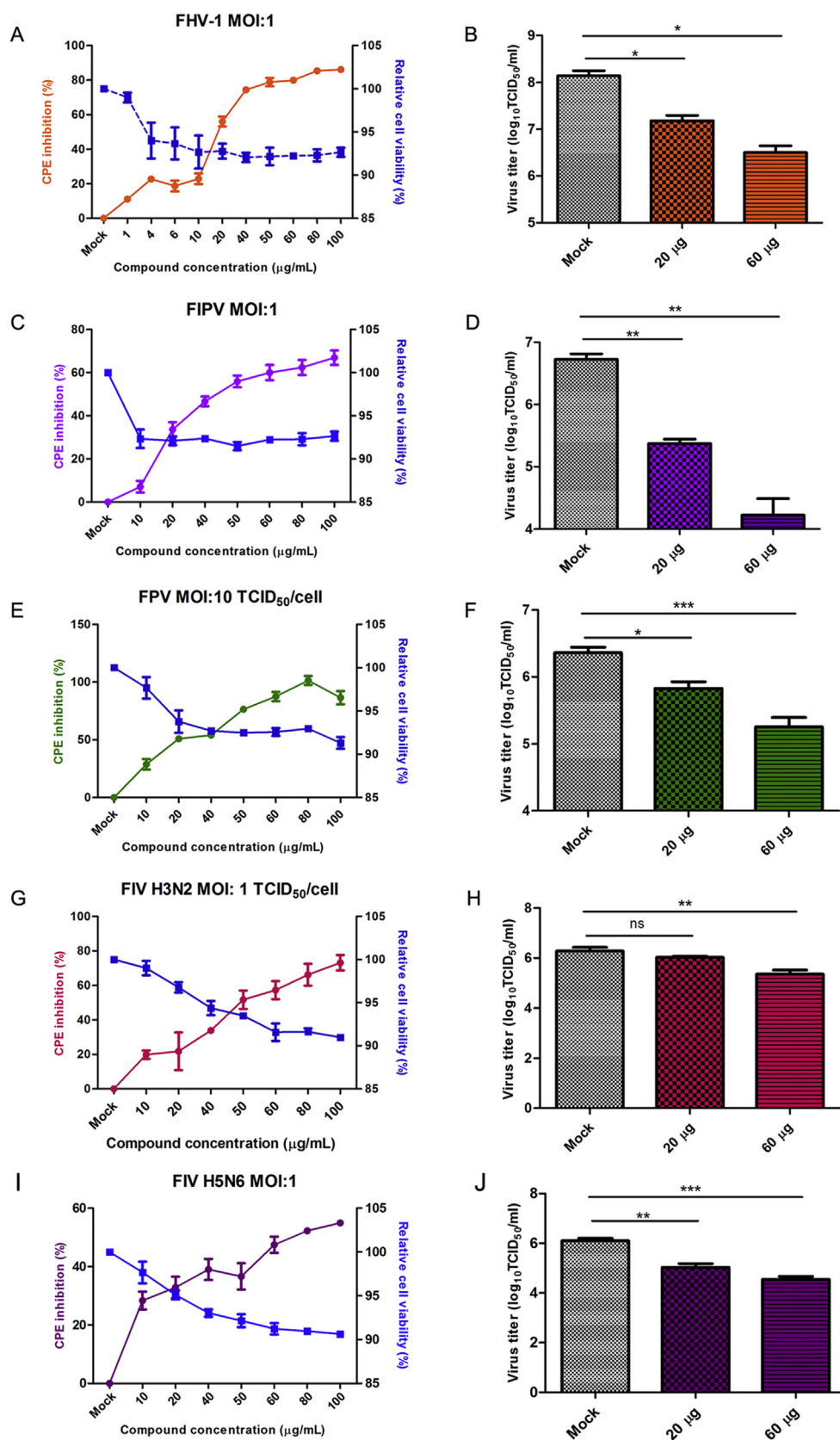
Fig. 4. Broad-spectrum antiviral activity test for IOP.

CRFK cells (10^4) (A–H) or MDCK cells (10^4) (I–J) were inoculated with a virus solution at an indicated MOI containing different concentrations of IOP (10, 20, 40, 50, 60, 80 and 100 μg/mL). CPE inhibition assay based on the resazurin (A, C, E, G, I) was performed at 48 hpi for FHV, at 72 hpi for FCoV, FPV and feline influenza virus (FIV) H3N2 and at 36 hpi for FIV H5N6. Cell viability was determined using a CCK8 assay. (B, D, F, H, J) Virus yield reduction in the cell supernatants from the control, 20 μg/mL IOP and 60 μg/mL IOP treated groups was determined in CRFK cells (B, D, F, H) or MDCK cells (J). Results represent the mean of three replicates ± SD, and each experiment was repeated three times.

Table 1
Viruses used in the experiment.

Viruses	Strain/Type	Group	Family	Location of isolation or purchase	Passage
FCV	F9	(+) ssRNA	Caliciviridae	USA	P3
	WZ-1			Harbin	P2
	XH			Harbin	P2
	HRB-SS			Harbin	P2
	TIG-1			Harbin	P2
	MD			Harbin	P2
	2280			ATCC	P2
FHV	VR-814	dsDNA	Alphaherpesvirinae	ATCC	P2
FPV	VR-648	ssDNA	Parvovirus	ATCC	P2
FIPV	VR-2004	(+) ssRNA	Coronaviridae	ATCC	P2
FIV	H5N6/H3N2	(−) ssRNA	Orthomyxoviridae	Harbin	P2

Table 2
Viruses tested for their susceptibility to IOP.

Viruses	Strain/Type	IC_{50} (µg/mL)	CC_{50} (µg/mL)	Host cell
FHV	VR-814	18.15 ± 1.5	>100	CRFK
FIPV	VR-2004	22.87 ± 1.1	>100	CRFK
FPV	VR-648	45.33 ± 3.2	>100	CRFK
FIV	H3N2	48.51 ± 2.8	>100	CRFK
	H5N6	68.47 ± 4.3	>100	MDCK

The data is an overview of cell-based experiments to evaluate the antiviral efficacy (IC_{50}) and overall toxicity (CC_{50}) of IOP. The antiviral activity of IOP was detected using multiple and unrelated viruses and on different cell types.

recombinant FCV and FHV that expresses eGFP was used, and IOP treatment reduced the fluorescence intensity after infection in vitro. The time-of-addition experiment performed with FCV suggested that the early stage of viral replication is affected by IOP. Because a variety of different virus families were inhibited by IOP, it appears likely that a potential cellular factor that is essential for viral replication was activated. However, we found that IOP treatment induces its inhibitory actions directly on virus particles and may affect viral binding/absorpting to cells directly.

Previous clinical animal experiments demonstrate that IOP may be a potential drug to prevent and cure cancer, cardiopathy, diabetes, AIDs, pancreatitis and other diseases [9–11]. Here, we described the broad-spectrum antiviral effects of IOP towards multi-feline viruses such as feline calicivirus (FCV), feline herpesvirus 1 (FHV-1), feline influenza virus (FIV), feline panleukopenia virus (FPV), feline coronavirus (FCoV). Moreover, IOP showed low cytotoxicity, and the CC_{50} was determined to be > 1 mg/mL. The IC_{50} for the viruses in this study ranged from 10 µg/mL to 100 µg/mL. Notably, IOP treatment shows no toxic effects on mouse major organs such as the liver, as evaluated by histopathological examinations [23]. These data indicate that IOP might be a potential feline antiviral agent due to its broad-spectrum antiviral activity and low toxicity.

IOP causes a default of viral entry, it is possible that the IOP acts on the virions and/or the cell receptor(s). The feline viruses investigated are members of RNA and DNA viral families, many of which do not use the same entry mechanism. A previous study indicated that the extracts of Ulva fasciata (U1) can inhibit JEV infection by forming the U1-JEV complex, then blocking virus adsorption [22]. Based on the investigation of the mechanism of IOP against FCV infection, only two hours of pre-treatment with IOP for cells before infection did not contribute to their resistance to FCV infection, suggestting that hose factors activated by IOP may not play important role in anti-FCV infection. However, both treatment of addition of IOP into the virus before infection or without pretreatment could inhibit FCV replication. So we speculate IOP can absorb these virus particles, which may prevent these viruses from entering into cells.

In summary, the broad-spectrum antiviral activity of IOP is identified. IOP potently inhibits at least five different families including RNA viruses (Caliciviridae, Coronaviridae and Orthomyxoviridae) and DNA viruses (Alphaherpesvirinae and Parvovirus). Further studies

are required to investigate the in vivo antiviral efficacy of IOP treatment.

Conflict of interest

The authors declare no financial or commercial conflicts of interest.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijbiomac.2016.11.054.

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