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ORIGINAL ARTICLE

Griffithsin inhibits porcine reproductive and respiratory syndrome virus infection *in vitro*

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Abstract

Porcine reproductive and respiratory syndrome virus (PRRSV) is a pathogen that severely disrupts swine production. Despite sustained efforts, the disease is still endemic, with high mortality and morbidity. New antiviral strategies to control PRRSV are needed. Griffithsin, a red algal lectin, has potent antiviral effect on several human enveloped viruses, but this effect has not been demonstrated on PRRSV. Here, we first tested the *in vitro* antiviral activity of Griffithsin against PRRSV. Griffithsin exerted strong saccharide-dependent antiviral activity against PRRSV, probably through interactions with glycans on the surface of PRRSV that interfered with virus entry. Furthermore we revealed that Griffithsin's action on PRRSV involved blocking viral adsorption, and it had no effect on viral penetration. Besides Our findings also suggested that Griffithsin may interfere with cell-to-cell spread to prevent virus transmission. The remarkable potency profile of Griffithsin supports its potential value as an antiviral agent against PRRSV.

Introduction

Infection by porcine reproductive and respiratory syndrome virus (PRRSV) causes devastating disease in swine, resulting in significant economic losses estimated at $644 million per year in the United States [1]. PRRSV is an enveloped, single-stranded positive-sense RNA virus. Its 15.4 kb genome contains at least 10 open reading frames [2], four of which encode envelope glycoproteins designated GP2a, GP3, GP4 and GP5. GP5 has been proposed as the major mediator of PRRSV entry into target cells.

PRRSV infection can cause respiratory distress, reproductive dysfunction and weight loss. Moreover, PRRSV infection confers susceptibility to secondary infection by other viruses or bacteria. Vaccination is the most common prophylactic measure used to protect pigs against PRRSV infection. Currently, modified live virus (MLV) PRRSV vaccines are considered most effective although probably because of PRRSV genetic variation, MLV vaccines have failed to confer complete protection against drift variants [3, 4]. Thus, there is a need for novel antiviral drugs that could be used for prevention and control of PRRSV infection.

Griffithsin, derived from *Griffithsia* spp. marine red algae., is a small lectin consisting of 121 amino acids [5]. Griffithsin is a domain-swapped dimer and each subunit has

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three nearly equivalent glycan-binding sites [6]. Griffithsin binds glycan moieties associated with the glycoproteins of several enveloped viruses [7], resulting in inhibition of infectivity. Griffithsin has been shown to exhibit significant antiviral activity against human enveloped viruses including HIV [5, 8], Middle East respiratory syndrome coronavirus (MERS-CoV) [9], severe acute respiratory syndrome corona virus (SARS-CoV) [6, 10], hepatitis C virus (HCV) [11, 12], herpes simplex virus 2 (HSV-2) [13] and Japanese encephalitis virus (JEV) [14, 15]. Moreover, Griffithsin shows excellent thermostability [16], remaining stable up to 80°C, and is resistant to organic solvents [5] and protease degradation [17]. The cytotoxicity of Griffithsin has been studied extensively [18, 19], showing that it possesses a superior safety profile: no cytotoxicity was observed against a variety of cell types, nor any major effects on peripheral blood mononuclear cell activation or cytokine and chemokine production. Thus, Griffithsin is an attractive candidate for development as an antiviral therapeutic.

In this study, we assessed the antiviral activity of Griffithsin against PRRSV in Marc-145 cells. Our results revealed that Griffithsin could effectively reduce PRRSV infection by blocking virus adsorption, indicating that Griffithsin may be a promising antiviral agent for treatment of PRRSV.

Materials and methods

Cells and viruses

Marc-145 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, USA) supplemented with 10% fetal bovine serum at 37°C under an atmosphere of 5% CO_2. Highly pathogenic PRRSV strain NJ-a (type 2) [20] was isolated, identified and stored in our laboratory at National Research Center of Engineering and Technology for Veterinary Biologicals, Jiangsu Academy of Agricultural Science. NJ-a strain was propagated in Marc-145 cells and viral stocks were titered to determine their $TCID_{50}$.

Protein expression and purification

A codon-optimized gene encoding Griffithsin (GenBank accession no. FJ594069) C-terminally tagged with His_6 was synthesized and cloned into the pUC57 vector using GenScript (Nanjing, China). Then recombinant plasmid pUC57-Griffithsin was introduced into the *Nde*I–*Xho*I digested pET-32a vector to obtain the recombinant expression plasmid pET-32a-Griffithsin. Competent *E. coli* BL21 (DE3) cells were transformed with the recombinant expression plasmid. Gene expression was carried out as described in the pET system manual (Novagen, USA). Briefly, a single colony from the recombinant *E. coli* strains was grown overnight at 37 °C in LB broth containing 100 μg/mL ampicillin and 20 μg/mL chloramphenicol. Then The overnight cultures were diluted in 400 mL of fresh LB broth at 37 °C until the optical density at 600 nm reached 1.0. Protein expression was induced with 1 mM isopropyl-beta-D-thiogalactopyranoside (IPTG). After further incubation for 24 h at 15 °C, the culture was harvested and resuspended in PBS. Then the cells were disrupted by high-pressure homogenization for two cycles. Finally, the cell lysates were centrifuged at 12000×g for 20 min at 4 °C and the resulting crude extract containing Griffithsin was applied to a 5 mL Ni^{2+}-Sepharose HisTrap HP™ affinity column (GE Healthcare, Sweden) and eluted with 300 mM imidazole. The protein was dialyzed and further purified by endotoxin removal and filter sterilization, then quantified using a BCA Protein Assay Reagent Kit (Pierce, USA). SDS-PAGE was used to analyze protein production.

Circular dichroism (CD) analysis of secondary structure

CD measurements were recorded at 25°C using a JASCO J-1500 spectropolarimeter. Purified Griffithsin was diluted to 200 μg/mL in 0.01 mM phosphate buffer, pH 7.2. Spectra were measured from 250 nm to 197 nm in a quartz cell with 0.1 cm path length, with a scanning speed of 100 nm/min and a data pitch of 1.0 nm. Phosphate buffer was used as a blank. The relative proportions of α-helix, β-sheet, β-turn and disordered conformation were analyzed using the Spectra Manager™ software platform.

Detection of Griffithsin by enzyme-linked immunosorbent assay (ELISA)

Binding of Griffithsin to glycans was evaluated by ELISA as previously described. [21, 22]. Briefly, flat-bottom 96-well microtiter plates (Nunc, Maxiscorp, MD, USA) were coated with 10 μg of ovalbumin (OVA). The plates were then washed with PBS and blocked by 3% (w/v) bovine serum albumin (BSA). Either Griffithsin alone or Griffithsin pre-incubated with 100 mM D-(+)-glucose or D-(+)-mannose was added to the wells. The wells only contained the BSA was as a blank. An anti-His_6 antibody (Boster, Wuhan, China) and horseradish peroxidase (HRP)-conjugated secondary antibody (Boster, Wuhan, China) were used to detect Griffithsin binding. After developing with 3,3',5,5'-tetramethylbenzidine (TMB) substrate, the reaction was stopped with 2 M H_2SO_4 and plates were read using a microplate spectrophotometer (BioTec) at a wavelength of 450 nm.

Cell viability assay

To evaluate the cytotoxicity of Griffithsin, Marc-145 cells in 96-well plates were treated for 48 h with different

concentrations of Griffithsin ranging from 0 to 5 μg/mL, and viability was measured using MTT assay. MTT was added to wells and incubated for 4 h. After removing the supernatant, dimethyl sulfoxide (DMSO) was added to each well for 10 min to dissolve the crystals and the absorbance at 490 nm was measured.

Inhibition of PRRSV by Griffithsin

Increasing concentrations of Griffithsin were pre-incubated with PRRSV at room temperature for 30 min, and then the virions were used to infect cells at a multiplicity of infection (MOI) of 5 and 10 for 30 min at room temperature. For competition assays, Griffithsin was pre-incubated with 100 mM D-(+)-mannose before incubation with PRRSV. Subsequently, the mixture was transferred to Marc-145 cells in 96-well plates. Uninfected cells served as a negative control, and cells infected with PRRSV served as a positive control. The cells were cultured at 37°C for 1 h and washed with PBS to remove unbound virus. Fresh medium was added and the cells were cultured for 24 h.

The presence of PRRSV was further assessed by indirect immunofluorescence assay. Cells were fixed with precooled methanol for 10 min at room temperature, blocked with PBS containing 3% (w/v) bovine serum albumin (BSA) for 3 h at 37°C, and then incubated with anti-PRRSV GP5 protein monoclonal antibody (TONGDIAN, Hangzhou, China) diluted 1:1000 in 1% BSA/PBS for 1 h at 37°C. After washing three times with PBS, the cells were incubated with FITC-conjugated goat anti-mouse IgG antibody (Boster,Wuhan, China) diluted 1:50 for 1 h at 37°C in the dark. After washing, the cells were stained with DAPI for 10 min at room temperature, washed again, and samples were observed using a Cytation™ 5 Cell Imaging Multi-Mode Reader (BioTek).

For quantitation of infected cells, three fields were randomly collected and processed for quantification using automatic cell counting software (Imaris 7.2.3). The results of quantitation were expressed as the percentage of infected cells.

Real-time quantitative PCR (RT-qPCR)

Total RNA was extracted from samples using a MiniBEST Universal RNA Extraction Kit (TaKaRa, Osaka, Japan) and converted to cDNA using PrimeScript™ Reverse Transcriptase. RT-qPCR was performed with a LightCycler 480 II System (Roche, Basel, Switzerland) using the EvaGreen 2× qPCR MasterMix-No Dye kit (abm, Canada) according to the manufacturer's recommendations. The forward primer (FP) and reverse primer (RP) for PRRSV and GAPDH were as follows: PRRSV-FP: 5′-TCTGGACACTAAGGGCAG ACTC-3′, PRRSV-RP: 5′-GGAACCATCAAGCACAAC TCTC-3′, GAPDH-FP: 5′-CCTTCCGTGTCCCT ACTGCC AAC-3′, GAPDH -RP: 5′-GACGCCTGCTTCACCACC TTCT-3′. Data were analyzed using the $2^{-\Delta\Delta Ct}$ method. Each assay was performed in triplicate.

Western blot analysis

Cells were lysed in cell lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.1 mM phenylmethylsulfonyl fluoride). Protein content was measured using the BCA Protein Assay Reagent Kit (Pierce, Rockford, IL, USA) and 20 μg total protein was loaded on a 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) gel, transferred to a polyvinyl difluoride (PVDF) membrane and blocked with TBS containing 5% skim milk and 0.1% Tween-20 at 37°C for 2 h. Membranes were incubated overnight with gentle shaking at 4°C with mouse anti-PRRSV GP5 antibody (1:500, TONGDIAN, Hangzhou, China) or mouse anti-β-actin antibody (1:200, Boster, Wuhan, China). After washing, membranes were incubated with HRP-conjugated goat anti-mouse IgG (1:5,000, Boster, Wuhan, China) for 1 h at 37°C. Antibodies were visualized using an enhanced chemiluminescence reagent and images were recorded using a ImageQuant LAS 4000 instrument (GE Healthcare, Sweden).

Effect of Griffithsin on PRRSV entry stages

Subconfluent monolayers of Marc-145 cells in 6-well plates were washed with cold PBS followed by DMED and then were pre-chilled at 4°C for 30 min. In adsorption assay, cells were incubated with PRRSV (MOI = 5) in the presence or absence of Griffithsin at 4°C for 1 h. Griffithsin pre-incubated with 100 mM D-(+)-mannose was served as a control. After washing with cold PBS to remove unbound virus, cells were harvested for viral genomic RNA detection using RT-qPCR as described above; In penetration assay, cells were infected with PRRSV at an MOI of 5 and incubated at 4°C for 1 h. After washing with cold PBS, some cells were treated with Griffithsin and shifted to 37°C for 1 h allowing for viral penetration. After removing Griffithsin, cells were also harvested for RT-qPCR detection as above description. described above

Griffithsin agglutination assay

Red blood cells collected from avian, swine, goat, guinea pig and mice were washed and resuspended in PBS at a final concentration of 1% (v/v). Twenty-five μL of Griffithsin (serially diluted two-fold) were mixed with an equal volume of erythrocytes in a 96-well v-bottom plate. The plate was incubated at room temperature for 30 min and results

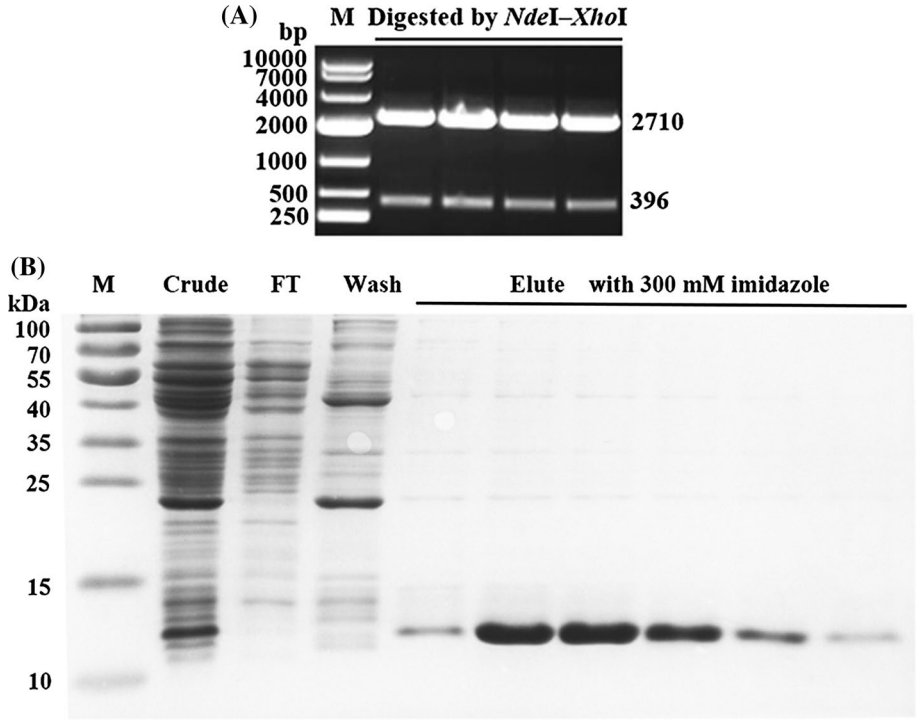

Fig. 1 Identification of recombinant plasmid pUC57-Griffithsin and SDS-PAGE analyses of Griffithsin. (A) Recombinant plasmid pUC57-Griffithsin was digested by *Nde*I–*Xho*I and verified in 1% Agarose Gel. (B) Recombinant Griffithsin was purified by Ni^{2+}- Sepharose HisTrap HP^{TM} affinity column by the analysis of Crude extract (Crude), Flow-through after loading crude extract on affinity column (FT), Fraction obtained by washing with 50 mM imidazole (Wash) and Fractions eluted with 300 mM imidazole.

were recorded using a Gel Image System (Tanon, Shanghai, China).

Data analysis

Data were presented as the mean ± standard deviation of three independent experiments. Differences between group means were analyzed using the Student's *t*-test with GraphPad Prism 6 software. Statistical significance was assumed when $P<0.05$.

Results

Secondary structure analysis using CD spectroscopy

The recombinant plasmid pUC57-Griffithsin was verified by restriction enzyme digestion (Fig. 1A) and recombinant Griffithsin was successfully expressed in *E. coli* and readily purified to homogeneity (Fig. 1B). To assess whether Griffithsin was well folded, we first studied its secondary structure using CD spectroscopy. The CD spectrum of Griffithsin displayed a maximum at 220.5 nm and a minimum at 199.5 nm (Fig. 2) which was consistent with a structure composed mainly of β-sheets. As shown in Table 1, Griffithsin secondary structure consisted mostly of β-sheets (51.1%) with roughly equal proportions of a-helices and turns.

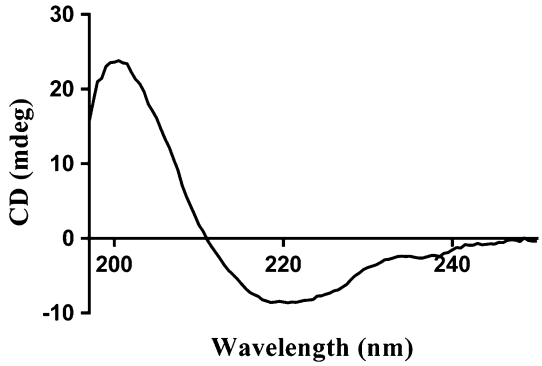

Fig. 2 CD spectrum of recombinant Griffithsin in 0.01 mM phosphate buffer, pH 7.2

Table 1 Secondary structure composition of recombinant griffiths in determined from its CD spectrum in the far-ultraviolet region at 25°C

Sample	a-helix (%)	β-sheet (%)	β-turn (%)	Random coil (%)
Recombinant griffithsin	23.1	51.1	21.6	4.3

Assessment of Griffithsin binding to OVA

The ability of Griffithsin to bind glycans was assessed by ELISA against immobilized OVA. OVA contains a single N-linked glycosylation site, in which bind to high-mannose and hybrid N-linked glycans have been characterized.

ELISA measurements revealed that Griffithsin interacted with OVA in a dose-dependent manner. OVA binding by Griffithsin was efficiently inhibited by 100 mM mannose and slightly less efficiently inhibited by 100 mM glucose, indicating that Griffithsin bound mannose with higher affinity compared with glucose (Fig. 3).

Griffithsin inhibits PRRSV infection *in vitro*

Immunofluorescence microscopy revealed that Griffithsin potently inhibited PRRSV infection of Marc-145 cells. As shown in Fig. 4.A, Griffithsin significantly decreased PRRSV infectivity in a dose-dependent manner, and this inhibition was substantially diminished in the presence of 100 mM mannose. Furthermore, the infection rate of 95.1% in the presence of 0 μg/mL Griffithsin, was reduced to 25.3% in the presence of 4 μg/mL Griffithsin when Marc-145 cells were infected with PRRSV at a MOI of 10 (Fig. 4.B). More significant reduction was observed when the cells were infected with 5 MOI PRRSV, where infection rate was reduced to 4.4% from 81.3% in the presence of 4 μg/mL Griffithsin (Fig. 4.B).

To understand whether these experimental results might instead be explained by a cytotoxic effect of Griffithsin, we assessed whether Griffithsin affected the proliferative activity of Marc-145 cells. As shown in Fig. 5, cells cultured in medium containing Griffithsin at the same concentrations as used in this study retained 100% viability compared with control cells.

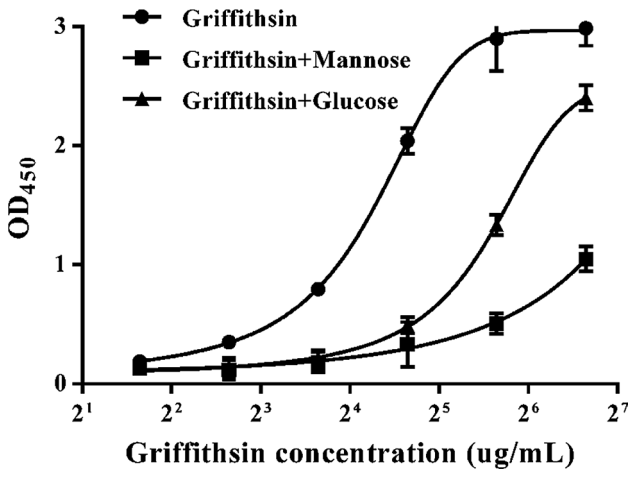

Fig. 3 ELISA binding of Griffithsin (circles), Griffithsin pre-incubated with mannose (squares) or Griffithsin pre-incubated with glucose (triangles) to OVA.

Griffithsin prevents the adsorption stage of PRRSV infection

To elucidate the mechanism of the antiviral effects of Griffithsin, a virus entry assay was performed. The data indicated that Griffithsin greatly repressed virus adsorption to cells, with no effects on virus penetration.

Marc-145 cells were incubated with PRRSV and Griffithsin at 4°C in the presence or absence of mannose to allow virus adsorption but not penetration. As shown in Fig. 6, 4 μg/mL Griffithsin greatly repressed PRRSV adsorption but had no effect on virions treated with Griffithsin in the presence of mannose ($P = 0.03$). No significant impact on PRRSV infectivity was observed when Griffithsin was added during viral penetration ($P > 0.05$).

Effects of Griffithsin on viral load measured using RT-qPCR and western blot

RT-qPCR was performed to investigate the effect of Griffithsin on intracellular viral load. Marc-145 cells were incubated with PRRSV at a MOI of 5 for 24 h, and then growth media was then replaced with fresh medium containing 4 μg/mL Griffithsin. Abundance of PRRSV GP5 RNA in Marc-145 cells was analyzed at different times post-Griffithsin treatment. As shown in Fig. 7.A, compared with the corresponding untreated control group, PRRSV RNA levels in the Griffithsin-treated group showed the highest decreases at 12 h ($P < 0.001$), 24 h ($P < 0.001$), and 36 h ($P < 0.001$). However, no significant differences in RNA abundance were observed at 6 h post-Griffithsin addition ($P > 0.05$). We also measured levels of PRRSV GP5 protein in Marc-145 cells using western blot after 36 h Griffithsin treatment. The results showed that expression of PRRSV GP5 was significantly reduced following Griffithsin treatment in a dose-dependent manner (Fig. 7.B).

Agglutination of Griffithsin

Since Griffithsin belongs to a group of lectins that potentially induce hemagglutination, so we determined whether Griffithsin had any hemagglutination activity. We tested blood cells derived from several species including avian, swine, goat, guinea pig and mice, and the results are shown in Fig. 8. The red cells of most species ' red blood cells were not agglutinated by Griffithsin. with the exception of guinea pig red blood cells, which were agglutinated in the presence of Griffithsin when used at a concentrations higher than 12.5 μg/mL.

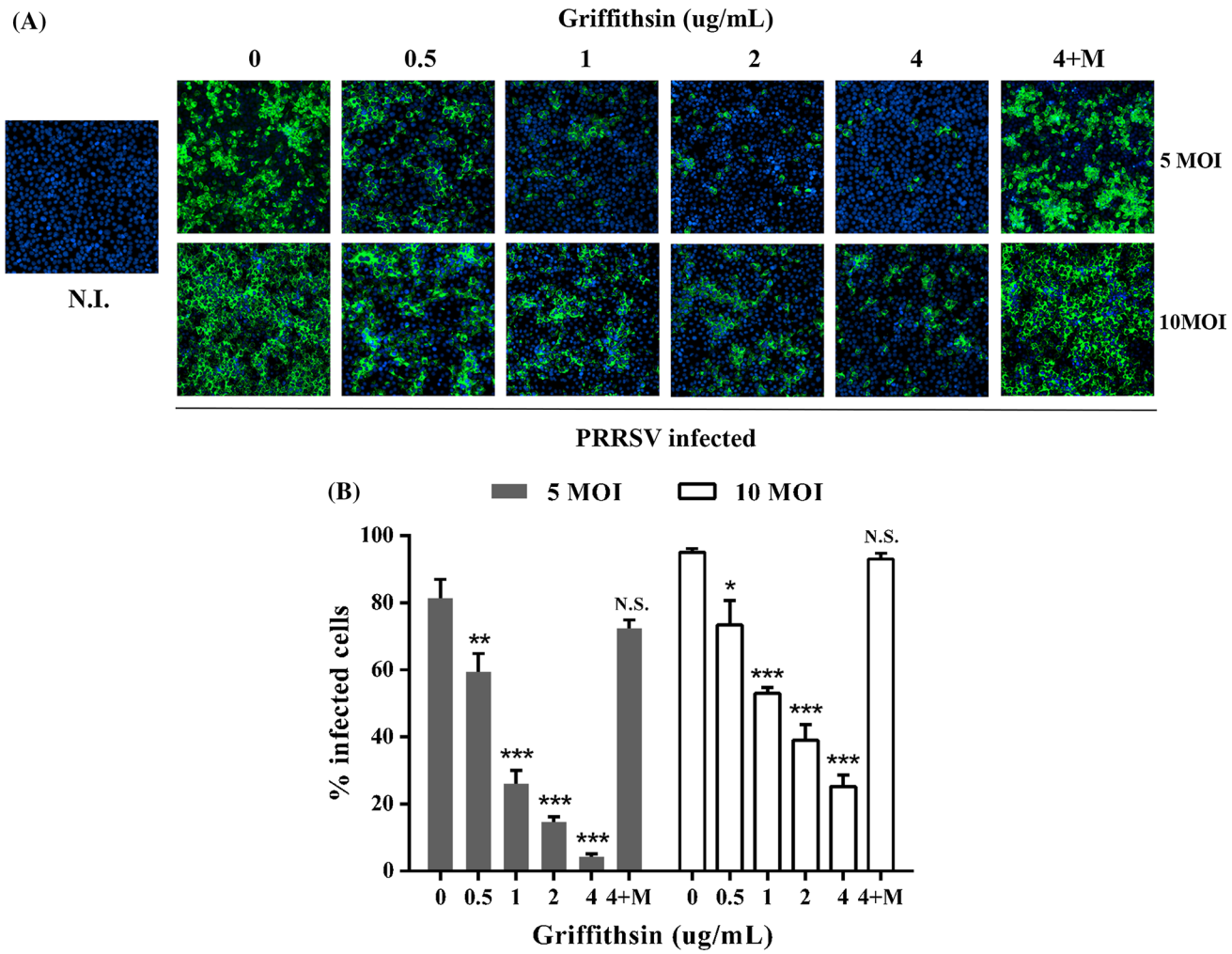

Fig. 4 Griffithsin inhibits PRRSV infection of Marc-145 cells. (A) Indirect immunofluorescence assay of PRRSV-infected Marc-145 cells in the presence of Griffithsin. (B) Quantification of PRRSV infected cells with the treatment of Griffithsin. The results are expressed as percent-infected cells calculated from the number of infected cells (FITC stain) / total number of cells (DAPI nuclei stain) × 100 %. Each value represents the mean of three independent experiments and its standard derivation. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to the 0 μg/mL control group.

Discussion

Griffithsin is currently considered the most potent anti-HIV agent for blocking virus entry by interacting with with the Env glycoprotein on the surface of HIV, exhibiting antiviral activity at concentrations in the nanomolar to picomolar range [8]. However, the antiviral activity of Griffithsin against PRRSV has not been investigated . PRRSV, like HIV, is an enveloped virus. and its major envelope glycoprotein, GP5, is heavily glycosylated [23, 24]. We analyzed therefore the potential ability of Griffithsin to inhibit infection by PRRSV, with the hope of developing a novel antiviral strategy for PRRSV therapy.

To further study its antiviral activity against enveloped viruses of veterinary importance, biologically active recombinant Griffithsin was produced in *E. coli* with high expression yields. Our data indicated that the major secondary structure elements of Griffithsin were β-sheets, consistent with previous reports [6, 25], indicating that its overall fold was probably similar to that of natural Griffithsin. Additional ELISA experiments demonstrated that the glycan-binding ability of Griffithsin, which was critical for its antiviral activity and further evaluated the anti-PRRSV activity of Griffithsin.

Our results showed that Griffithsin possessed potent antiviral activity against PRRSV in Marc-145 cells. Previous studies have been reported that certain lectins like porcine SP-A are able to inhibit inhibited PRRSV infection [26–30]. . Interestingly, Griffithsin was found to reduce PRRSV infectivity of Marc-145 cells at a 7.5-fold lower concentrations and at a 500-fold higher MOI compared with porcine SP-A [20]. Porcine ficolin (10 μg/mL) which

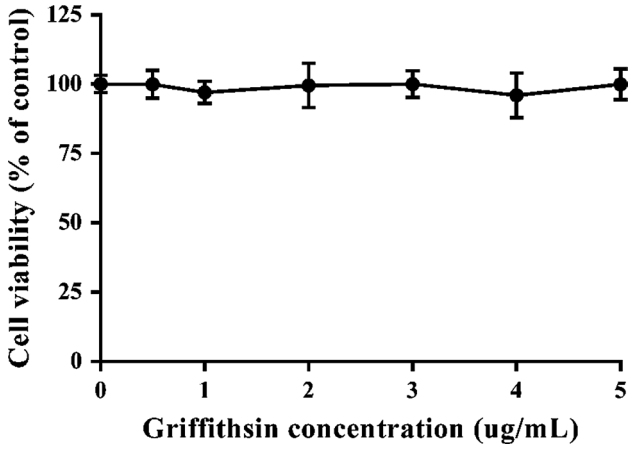

Fig. 5 Cytotoxicity of Griffithsin in Marc-145 cells was measured using the MTT assay. Cells were incubated with different concentrations of Griffithsin for 48 h, and then the cell viability assay was performed.

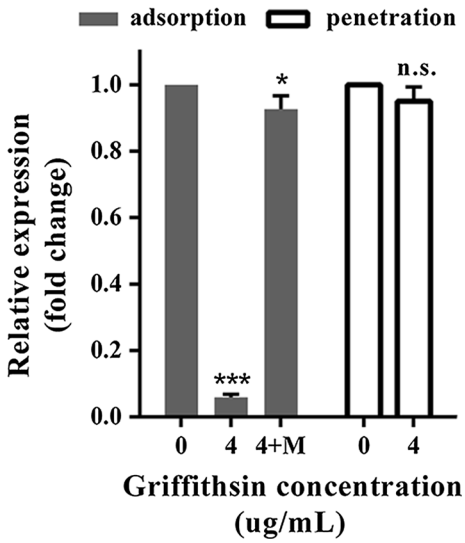

Fig. 6 Effect of Griffithsin on PRRSV entry stages. In control group, cells were infected with PRRSV without Griffithsin treatment at any stage. Data are presented as means ± standard deviations of three independent experiments. $*P < 0.05$;$**P < 0.01$; $***P < 0.001$ compared to the control group.

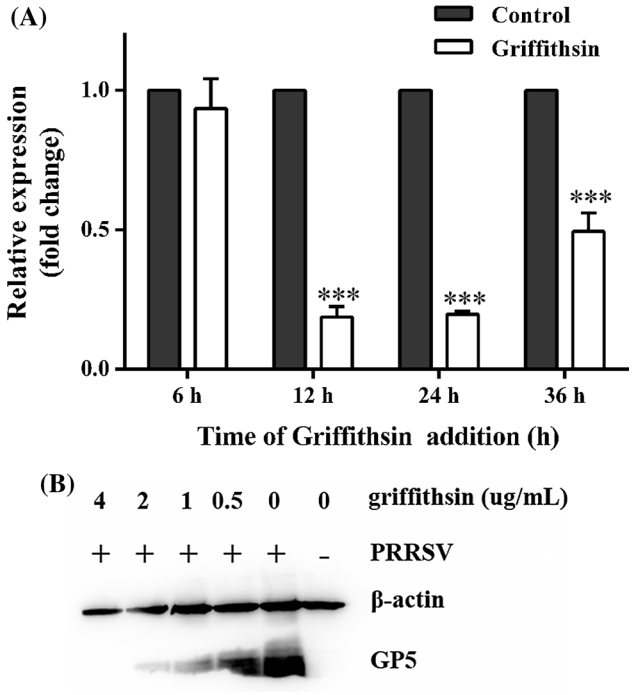

Fig. 7 Influence of Griffithsin on total PRRSV RNA and protein levels in Marc-45 cells. (A) Level of PRRSV RNA after treatment was determined by RT-qPCR at different time points post-infection with Griffithsin-treated virions. Relative expression (fold change) in comparison with a control group not treated with Griffithsin (denoted as 1) is illustrated. Data are presented as means ± standard deviations of three independent experiments. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ compared to the control group. (B) The PRRSV GP5 protein and β-actin were detected by western blot. β-actin was used as the internal control.

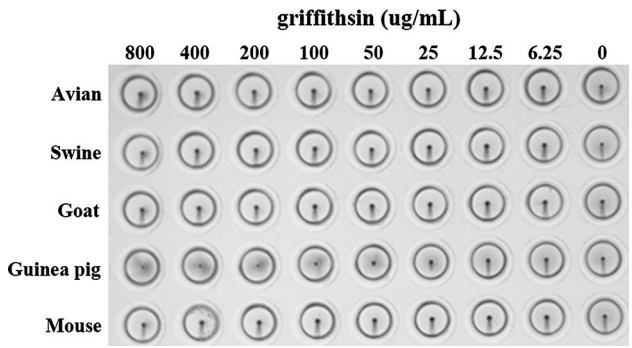

Fig. 8 Griffithsin hemagglutination activity. Avian, swine, goat, guinea pig and mouse erythrocytes were used to assess the hemagglutination activity of Griffithsin.

is also a lectin a lectin has also been shown to inhibit PRRSV infection [31]. However, our previous work demonstrated that solubility and low expression represented were the main obstacles to large-scale cost-effective production of biologically active ficolin. Finally, Although many other antivirals against PRRSV have been described in the literature, none have been able to effectively treat or prevent PRRSV infection *in vivo* for whatever reason .and Griffithsin may thus represent a candidate agent for preventing or treating PRRSV infection.

Another key finding of our study was that Griffithsin inhibited infection by PRRSV using a higher dose than for HIV strains, but at lower than that required those for SARS-CoV strains [7]. Since the HIV Env glycoprotein is heavily glycosylated containing up to 50% carbohydrate

by weight [8, 32], this may explain Griffithsin's potent anti-HV activity. The antiviral properties of Griffithsin against enveloped viruses is thus likely to be related to the glycosylation of viral proteins, a hypothesis that should be further investigated by further studies.

Most interestingly, our results clearly showed that Griffithsin was able to block virus adsorption but had only modest effects on virus penetration, which is consistent with most previous reports. Millet and colleagues performed detailed studies to define the viral life cycle stage at which Griffithsin acted and found that Griffithsin inhibited MERS-CoV by acting on the adsorption stage [9]. By contrast, Griffithsin exhibited its antiviral activity against HSV-2 by preventing cell-to-cell spread, but had little effect on HSV-2 entry into target cells [33]. The reason for this difference may be related to the virus itself and its entry receptors. Further studies are needed to clarify the anti-PRRSV activity of Griffithsin in porcine alveolar macrophages, the main targets of PRRSV infection *in vivo*.

Our data clearly showed that Griffithsin displayed its antiviral activity in a saccharide-dependent manner. Taking our own findings and the results of previous studies together, we inferred that the binding of Griffithsin to PRRSV glycoproteins prevented PRRSV attachment to target cells, possibly due to steric hindrance or loss of PRRSV receptor-binding ability. Previous studies revealed that Griffithsin reduced HIV, HCV, SARS-CoV, MERS-CoV, and JEV infectivity through binding to viral glycoproteins, thereby blocking viral entry. It was also confirmed that Griffithsin could bind to glycoprotein of HSV-2 and inhibited viral transmission by blocking cell-to-cell spread. Interestingly, Griffithsin also has antiviral activity against HPV, a non-enveloped virus, by binding to the secondary receptor α6 integrin and decreasing its availability on the cell surface [7]. Due to the uncertain nature of the interactions between Griffithsin, PRRSV and host cells, its mode of action should be clarified in future studies.

Our study also showed that Griffithsin greatly reduced total viral RNA 12 h, 24 h and 36 h post-infection, but had no effect at 6 h post-infection, after the initial rounds of viral replication. This finding suggested that Griffithsin may neutralize progeny virions and/or interfere with cell-to-cell spread, a possibility which needs to be studied by future work.

In conclusion, our study provides the first evidence of the antiviral activity of Griffithsin against PRRSV. Our results revealed that Griffithsin exerted a potent inhibitory effect on highly pathogenic PRRSV by interfering with viral entry and/or cell-to-cell spread. Thus, Griffithsin may seems to be a candidate agent for preventing PRRSV infection and further studies in live animals are necessary to confirm its value for inhibiting PRRSV infection *in vivo*.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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