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Research Article

Antiviral Activity of Recombinant Porcine Interferon-α Against *Porcine Transmissible Gastroenteritis Virus* in PK-15 Cells

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Abstract

A recombinant porcine interferon alpha (rPoIFN-α) has been developed and patented previously (Chinese patent number ZL200810020180.4). In the current study, we investigated the inhibitory effects of the rPoIFN-α on the propagation of *porcine transmissible gastroenteritis virus (TGEV)* at different doses in porcine kidney cell line (PK-15). To quantitatively determine the inhibition of viral growth by rPoIFN-α, TCID50 assay, plaque formation assay, real-time qRT-PCR, western blot and immunofluorescence assay were adopted to evaluate the changes of viral infectious particles, viral genome copy numbers and viral protein expression levels respectively. The results demonstrated that all the three batches of the rPoIFN-α tested inhibited *TGEV*-induced cytopathic effect in PK-15 cells with very similar potency. rPoIFN-α inhibited *TGEV* proliferation more strongly than human IFN-α product. The inhibitory activity of rPoIFN-α on *TGEV* growth in culture was dose dependent, and the activity was gradually reduced with the decreasing of the concentration of rPoIFN-α.

Keywords: *Recombinant porcine interferon-α (rPoIFN-α), Porcine transmissible gastroenteritis virus (TGEV), Immunofluorescence assay, Real-time qRT-PCR, TCID50 assay, Western blot*

PK-15 Hücrelerinde *Domuz Transmissible Gastroenteritis* Virüsüne Karşı Rekombinant Domuz İnterferon-α'nın Antiviral Aktivitesi

Özet

Bir rekombinant domuz interferon alfa (rPoIFN-α) geliştirilmiş ve patenti daha öncesinde alınmıştı (Çin patent numarası ZL200810020180.4). Bu çalışmada, rPoIFN-α'nın farlı dozlarının *domuz transmissible gastroenteritis virüs (TGEV)*'ün üremesi üzerindeki baskılayıcı etkisi domuz böbrek hücre kültüründe (PK-15) araştırıldı. rPoIFN-α ile viral büyümenin baskılanmasını kantitatif olarak belirlemek için TCID50 testi, plak oluşum testi, gerçek zamanlı qRT-PCR, western blot ve immunfloresan teknikleri viral enfeksiyöz partiküllerin değişimlerini, viral genom kopya sayılarını ve viral protein ekspresyon seviyelerini belirlemek amacıyla uygulandı. Araştırma sonuçları test edilen üç rPoIFN-α'nın da PK-15 hücrelerinde *TGEV* ile oluşturulmuş sitopatik etkisinin aynı derecede olduğunu göstermiştir. rPoIFN-α, *TGEV* proliferasyonunu insan IFN-α ürününden daha güçlü olarak inhibe etti. Kültürde *TGEV* büyümesine rPoIFN-α'nın baskılayıcı aktivitesi doza bağımlı olup aktivite rPoIFN-α'nın azalan dozu ile göreceli olarak azalma gösterdi.

Anahtar sözcükler: *Rekombinant domuz interferon-α (rPoIFN-α), Domuz transmissible gastroenteritis virüs (TGEV), İmmunfloresan tekniği, Gerçek zamanlı qRT-PCR, TCID50 testi, Western blot*

INTRODUCTION

Porcine transmissible gastroenteritis virus (TGEV) is an enveloped virus that contains a large, positive-sense single-stranded RNA genome, belonging to the genus of *Alphacoronavirus* in the family of *Coronaviridae* [1]. The genomic size of *coronaviruses* ranges from approximately 28.6 kilobases. *TGEV* causes transmissible gastroenteritis

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(TGE) in pigs, and its mortality is close to 100% in young pigs. This disease is the major infectious disease that restricts the healthy development of pig breeding industry and results in huge economic losses to animal husbandry [2]. At present, however, there is no vaccine available for *TGEV* infection. Although antiviral agents such as ribavirin may be used to treat *TGEV* infection, severe side effects that come with ribavirin have been found in piglets including the toxicity to erythrocytes, bone marrow cells, as well as the epithelial cells of the gastroentestine and pancreas, which greatly restricted its use in animals [3].

Interferons (IFNs) are cytokines that are crucial for preventing viral replication at the site of infection and for coordinating adaptive immune responses that lead to the development of long-lasting, specific immunity. IFNs are composed of three physiologically distinct types I, II, III [4]. IFN-α belongs to type I, which plays an important role in innate immunity against viral infections [5,6]. The antiviral activities of porcine IFN-α (PoIFN-α) have been widely observed in response to infections with *Foot-and-mouth disease virus* (*FMDV*) [7-9], *Porcine respiratory and reproductive syndrome virus (PRRSV)* [10,11], *Pseudorabies virus (PRV)* [12], *Vesicular stomatitis virus (VSV)* [13], *Classical swine fever virus (CSFV)* [14], and *Influenza viruses* (IFV) including the swine origin *influenza virus A (H1N1)* [15-17]. There have been a great deal of studies that demonstrated antiviral activity and adjuvant function of recombinant PoIFN-α in various models of infection, suggesting that recombinant PoIFN-α might be a potential antiviral agent for the control of swine virus infections [18-22]. Both human IFN-α and natural porcine IFN-α have been shown to have antiviral activity in response to *TGEV* infection in vitro [23,24]; however, to our knowledge, no detailed report on the anti-*TGEV* activity of recombinant PoIFN-α (rPoIFN-α) is available.

We have successfully produced rPoIFN-α with high biological activity (Chinese patent ZL200810020180.4). In order to investigate the inhibitory effects of this rPoIFN-α on the propagation of *TGEV* in PK-15 cells, we employed five different methods, including $TCID_{50}$ assay, plaque formation assay, real-time qRT-PCR, western blot and immunofluorescence assay, to analyze the inhibitory effect of rPoIFN-α on the proliferation of *TGEV*. We hope the data from this research could lay a foundation for clinical trials of rPoIFN-α.

MATERIAL and METHODS

Drugs, Cells and Virus

The rPoIFN-α in this study was produced by our team (Chinese patent number: ZL200810020180.4). Briefly, *PoIFN-α* gene was cloned into a prokaryotic expression vector pET32a, which was then transformed into *E. coli* BL21 (DE3) strain before IPTG was added to induce the expression of the recombinant protein. The product yielded was purified with a two-step chromatographic procedure (Ni^{2+} affinity chromatography and DEAE anion exchange chromatography), and its biological activity was achieved as high as 1.1×10^6 IU/mL.

Three batches (2013001, 2013002 and 2013003) of rPoIFN-α were included in the study. Their titers was 2.01×10^4 IU/vial, 2.06×10^4 IU/vial and 2.02×10^4 IU/vial, respectively. Human interferon standard (HuIFN, batch number 07/01, 1.1×10^4 IU/vial) was provided by the National Institute for the Control of Pharmaceutical and Biological Products of China. Pig kidney epithelia cell line (PK-15 cells, ATCC® CCL-33) was cultured in Dulbecco Minimal Essential Medium (D-MEM) (Gibco BRL, MD, USA) supplemented with 10% heat-inactivated newborn bovine serum (Gibco BRL, MD, USA), 100 µg/mL of streptomycin and100 IU/mL of penicillin, 2 mmol/L L-glutamine, 75 g/L $NaHCO_3$, pH 7.2. PK-15 cell suspension was adjusted to 1.0×10^5/ml and 0.1 ml was transferred to each well of a 96 well cell culture plate before the incubation at 37°C in a 5% CO_2 atmosphere incubator.

TGEV was gifted by Professor Zhi-Wen Xu (Sichuan Agricultural University, Yaan, Sichuan Province, China) and identified by the viral CPE in PK-15 cells, RT-PCR and sequence analysis. Viral titers were determined as 10^{-5} $TCID_{50}$/mL with the calculation formula of Reed and Muench [25].

TGEV Titration ($TCID_{50}$ Assay)

The inhibition effects of rPoIFN-α on the growth of *TGEV* were determined by the changes of *TGEV* titers in PK-15 cells. The cells were plated onto 96-well plates at 1.0×10^4/well followed by the incubation for 24 h in a 5% CO_2 atmosphere incubator at 37°C. When the cell monolayer reached to 90% confluency, the cells were infected with 100 $TCID_{50}$ *TGEV* and treated with two-fold serially diluted rPoIFN-α at 1 h post-*TGEV* infection. The antiviral activity of the rPoIFN-α was expressed as $TCID_{50}$ in PK-15 cells, defined as the amount of the virus that produces CPE in 50% of PK-15 cells inoculated. At the same time, normal cell control, the virus control, human interferon-α control were included in the experiment. $TCID_{50}$ was determined by the Reed-Muench method as previously described [26,27].

Plaque Assay

PK-15 cells in 6-well plate were pretreated with serially diluted rPoIFN-α and incubated at 37°C for 24 h in a 5% CO_2 atmosphere incubator. The culture medium was then removed and 100$TCID_{50}$ of *TGEV* in 100 µL were added to each well and incubated at 37°C for 1 h with 5% CO_2. After the culture was washed twice with PBS, agarose nutrient broth (DMEM containing 3% calf serum and 0.75% agarose) was added at 1 mL per well. The culture was further incubated at 37°C for 5 days with daily monitor and record of the plaque appearance time, shape/size and numbers. The total plaque numbers were counted after staining with crystal violet.

Quantification of TGEV by Real-time qRT-PCR after Application of rPoIFN-α

PK-15 cells (1.0×10^4/well) were pretreated with serially diluted rPoIFN-α at 37°C for 24 h in a 5% CO_2 incubator. The culture supernatant was removed, the cells were washed with PBS before 100$TCID_{50}$ *TGEV* in 100 µL was added and incubated at 37°C for 1 h. The culture was replaced with DMEM containing 2% heat-inactivated newborn bovine serum after washing twice with PBS. The plates were incubated at 37°C for 24 h in a 5% CO_2 incubator before total RNA was extracted with Trizol reagent (Invitrogen, Inc.). Each RNA sample was reverse transcribed using Reveraid First Strand cDNA Synthesis Kit (ThermoFisher, Waltham, MA, USA). Sequences of the PCR primers for the amplification of the 258 bp fragment of *TGEV* S gene were (forward) 5′-GTATTGGGATTATGCT-3′ and (reverse) 5′-CCACAATTTGCCTCTG-3′. The cycling condition was composed of 95°C for 5min, followed by 35 cycles of 95°C for 50 s, 48°C for 30 s, 72°C for 30 s and a final cycle at 72°C for 10 min. PCR product was cloned into pCR®-T easy vector (Invitrogen, Inc.). RNA fragments coding for *TGEV* S protein were prepared by in vitro transcription with the plasmid DNA as template. The concentration of the transcripts was determined by spectrophotometer (NanoDrop 2000, Wilmington, US) after the template DNA was removed. Standard curves for the qRT-PCR were generated using serial dilutions of the RNA fragments (within a range from 5-200 ng/µL) to convert Ct values into arbitrary values. These values were then normalized with the mean values of the house-keeping gene - porcine β-actin (forward primer 5′-GAGAAGCTGTGCTACGTCGC-3′ and reverse primer 5′-CCAGACAGCACTGTGTTGGC-3′) [28]. The copy number of the viral genome in the experimental samples was determined by interpolating the threshold cycle values using the standard curve. The qRT-PCR reactions were carried out in 20 µL volume containing dNTP, SYBR Green I (Roche, Basel, Switzerland), primers (0.2 µM each) and target cDNA. PCR amplification included an initial denaturation at 95°C for 5 min, followed by 45 cycles of denaturation at 95°C for 10 s, annealing at 56°C for 10 s, and elongation 72°C for 15 s. After the cycling was completed, a melting curve was constructed to confirm the authenticity of the amplified products. A negative control sample that contained no template RNA was run with each experiment.

Western-blot Analysis of rPoIFN-α Inhibition on TGEV Spike Protein Expression

PK-15 cells were pretreated with rPoIFN-α as described methods. The total cellular protein was extracted using Radio Immunoprecipitation Assay Lysis Buffer (Beyotime, Shanghai, China). Protein concentration was determined using BCA Protein Assay Kit (Beyotime, Shanghai, China). Fifty µg of protein were loaded and electrophoresed on 15% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE). Subsequently, the proteins were transferred to polyvinylidene difluoride (PVDF) membrane (Millipore Corp, Atlanta, GA, USA). The membrane was blocked with 5% nonfat milk at room temperature for 1 h, and then incubated with *TGEV* spike protein antibody (JBT-9181, Hannotech, Korea) overnight at 4°C followed by HRP-conjugated secondary antibodies at room temperature for 1 h. The signals in the membrane were detected using ECL reagent (ThermoFisher, Waltham, MA, USA).

Immunofluorescence Assay

PK-15 cells were cultured on cover slips and treated as indicated in methods. Cells were then fixed with 4% formaldehyde for 30 min, and incubated in blocking buffer (1% bovine serum albumin in PBS, 0.1% Triton-X100) for 1 h. Subsequently, the slides were incubated with anti-*TGEV* monoclonal antibody (Abcam, ab20301) overnight at 4°C, and then incubated with the FITC labeled anti-mouse IgG (Abcam, ab6785) for 1 h at room temperature. The slides were mounted and images were acquired by using a fluorescence microscope (OLYMPUS IX73, Japan).

Statistical Analysis

All data were presented as mean ±SEM from three independent experiments as triplicate. The results were analyzed by One-way analysis of variance (ANOVA) using the SPSS manager software (version 18.0, licence serial: 10034432, CODE:c66b5316e05ac32a8434). A value of $P<0.05$ was considered significant. $P<0.01$ was considered highly significant.

RESULTS

The Influence of rPoIFN-α to TGEV Titers

The inhibition effects of rPoIFN-α on *TGEV* proliferation were determined by the reduction of $TCID_{50}$ in PK-15 cells. We compared the antiviral effect of rPoIFN-α to that of human IFN-α standard by determining *TGEV* titers with the formula of Reed and Muench [25]. The results showed that the inhibition of rPoIFN-α on the multiplication of *TGEV* gradually reduced as the dose of rPoIFN-α in PK-15 cells was decreased from 1:2 to $1:2^8$ *(Fig. 1)*. The inhibition effect of human IFN-α was comparable to that of rPoIFN-α although the inhibition appeared not as well as rPoIFN-α on *TGEV* in PK-15 cells.

Plaque Formation Assay to Detect Changes on the Virus Numbers of TGEV Infections

The plaque formation assay was carried out with three different batches of rPoIFN-α lyophilized product, and the results are shown in *Fig. 2* and *Table 1*. It showed that all the 3 batches of rPoIFN-α demonstrated dose-depend

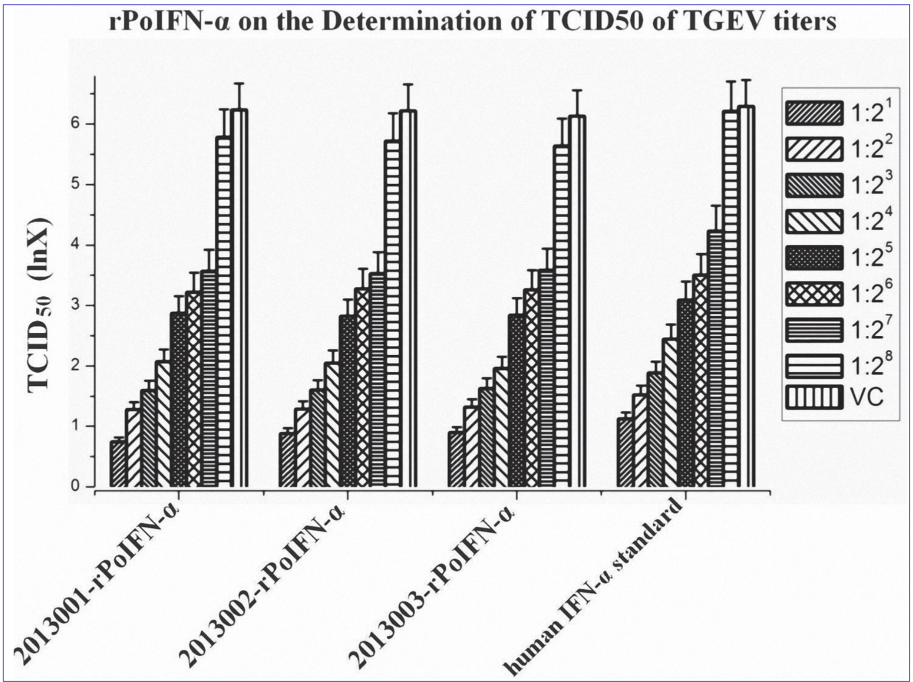

Fig 1. Different batch, different doses of rPoIFN-α on the Determination of $TCID_{50}$ of *TGEV* titers. Data are expressed as the average ± standard deviation (X±SD) which are shown above. "VC" means "virus control"

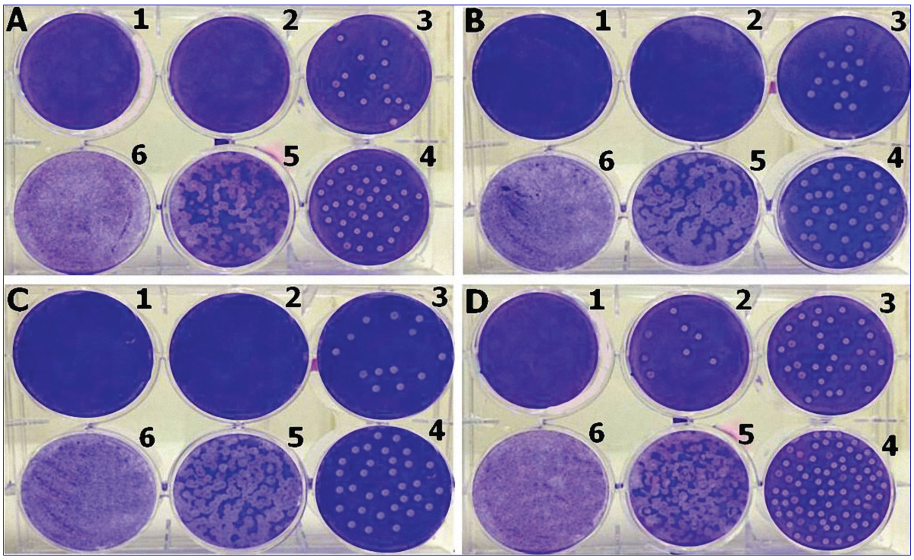

Fig 2. Plaque formation assay with different batches and doses of rPoIFN-α

A: rPoIFN-α batch 2013001. B: rPoIFN-α batch 2013002. C: rPoIFN-α batch 2013003. D: Human IFN-α standard. Wells 1: PK-15 cell control. Wells 2~5: With rPoIFN-α in the dilutions of 1:32, 1:64, 1:128 and 1:256. Well 6: $100TCID_{50}$ virus control

inhibition on the formation of virus plaques in PK-15 cells. Viral plaques started to appear when rPoIFN-α was diluted to $1{:}2^6$, and became too many to count when rPoIFN-α reached to $1{:}2^8$ dilution. The inhibitory effect of human interferon on *TGEV* was similar to that of rPoIFN-α, but the number of plaques was more than those with rPoIFN-α, which suggested that the effect on *TGEV* proliferation by rPoIFN was significantly higher than that of human interferon.

The Inhibition of rPoIFN-α on TGEV Multiplication by qRT-PCR Assay

As shown in *Fig. 3* and *Table 2*, the viral copy numbers decreased with the increase of rPoIFN-α dilution ratio, which suggested that rPoIFN-α had a significant inhibition effect on the multiplication of *TGEV*. The inhibition effect of human IFN-α was not as good as rPoIFN-α on *TGEV* in PK-15 cells.

Inhibition of rPoIFN-α on the Expression of TGEV Spike Protein by Western Blot

To evaluate rPoIFN-α as an inhibitor against *TGEV* replication, the expression of *TGEV* spike protein was investigated by western blot in *TGEV* infected PK-15 cells in which rPoIFN-α was diluted from 1:16 to 1:256. Inhibition with rPoIFN-α was more pronounced than that with human IFN-α. The highest inhibition level appeared at the dilution of 1:32 (P<0.01). As expected, the expression level of *TGEV* spike protein in the culture without rPoIFN-α treatment was the highest among all the samples *(Fig. 4A, 4B)*.

ImmunofluorescenceAssayfor Testing the Inhibition of rPoIFN-α to TGEV in vitro

In *Fig. 5,* it was shown that the number of *TGEV* fluorescence positive cells increases gradually with the increase of the dilution factor of the three rPoIFN-α products, indicating the inhibition effect of *TGEV* is gradually decreased when the dilution of rPoIFN-α exceeded 1:32.

DISCUSSION

IFNs are a group of cytokines, initially identified by their ability to induce resistance to viral infection, it is currently also recognized as pro-inflammatory molecules and potent modulators of both innate and adaptive immune responses. Recent studies have shown that IFNs play a key role in the immune response to *TGEV*. As An et al.[29] reported, *TGEV* infection induced interferon signal transducer and activator of transcription 1 STAT1 phos-

Table 1. *The results of plaque formation assay*

rPoIFN-α Dilution	The Numbers of Plaque			
	rPoIFN-α (2013001)	rPoIFN-α (2013002)	rPoIFN-α (2013003)	Human IFN-α Standard
2^1	0	0	0	0
2^2	0	0	0	0
2^3	0	0	0	0
2^4	0	0	0	0
2^5	0	0	0	8
2^6	14	16	13	30
2^7	34	35	32	75
2^8	N	N	N	N

N: *Too many plaques to count*

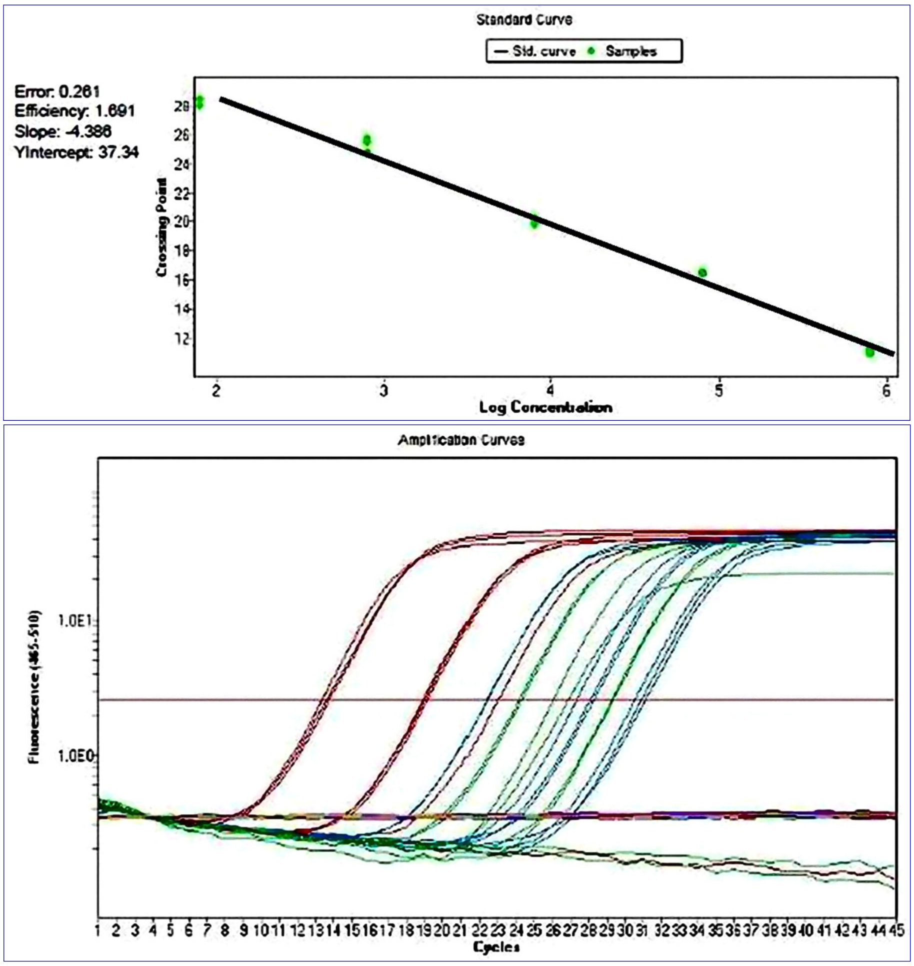

Fig 3. Standard curve (A) and copy numbers (B) of *TGEV* S gene determined by qRT-PCR
(A) standard curve of *TGEV* S gene real-time quantitative RT-PCR (B) detection results of copy numbers of *TGEV* S gene real-time quantitative RT-PCR

phorylation and nuclear translocation, as well as interferon-stimulated genes (ISGs) expression. Jordan et al.[30] found that titres of *TGEV* were reduced between 6 and 15 h post-infection in swine testis cells if the cells were treated with 1000 units/mL or 2500 units/mL of IFN. Lee et al.[31] demonstrated that the combined administration of the swIFN and swIL-18 cytokines using attenuated *Salmonella enterica serovar Typhimurium* as an oral carrier provided enhanced protection against intestinal tract infection with *TGEV*. Zhu et al.[32] modified rare codons encoding for 6 amino acids of porcine interferon-α and expressed the modified PoIFN-α gene in *Pichia pastoris*. The authors reported that the modified interferon-α showed more potent protection than that of the original protein in *VSV* or *TGEV* infected cells, the magnification factors reaching 100 for the *TGEV* and 300 for the *VSV*. The higher antiviral activities of the modified IFN-α gene was attributed to its higher expression and higher concentration of the cytokine.

IFN-α is encoded by a family of closely related intronless genes in all mammalian species [33]. They are mainly produced by virus infected peripheral blood leukocytes, or lymphoblastoid and myeloblastoid cell lines [34]. The porcine IFN-α (PoIFN-α) gene family is located on chromosome 1 [35]. Currently there are 17 different PoIFN-α subtypes (PoIFN α1-α17) with different antiviral activities and different expression profiles, among them PoIFN-α1 showed highest antiviral activity and anti-inflammatory activity at 10 IU/mL [36].

Proteins of PoIFN-*α* subtypes consist of 158 to 166 amino acid residues with monomer active form and most

***Table 2.** TGEV copy numbers with different batch of rPoIFN-α at different doses*

Dilution of rPoIFN-α	Copy Numbers of TGEV			
	2013001 Batch of rPoIFN-α	2013002 Batch of rPoIFN-α	2013003 Batch of rPoIFN-α	National Human Interferon-α Standard
2^1	3.94E0	7.34E0	1.27E0	8.67E0
2^2	1.53E2	3.67E2	5.32E2	6.58E2
2^3	1.01E3	9.43E2	1.00E3	9.98E2
2^4	9.74E3	7.90E3	9.45E3	7.24E3
2^5	9.91E4	5.43E4	9.55E4	1.56E4
2^6	4.50E5	8.87E4	7.07E5	9.86E5
2^7	4.58E5	1.47E5	5.51E5	9.29E5
2^8	8.02E5	5.74E5	9.81E5	6.05E6
NC*	0	0	0	0

** **NC:** negative controls*

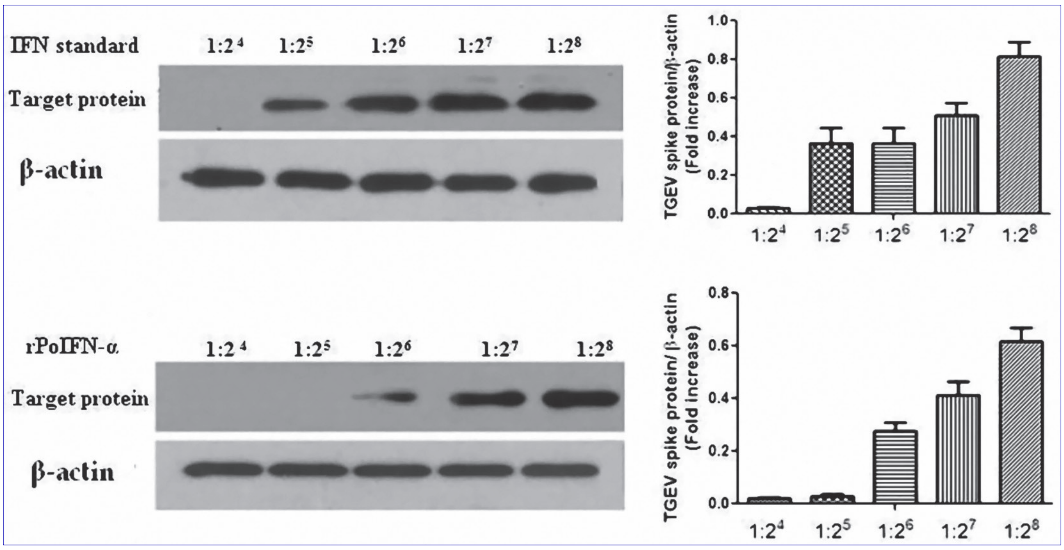

Fig 4. The expression of *TGEV* spike protein as Interferon standard and rPoIFN-α inducement for Serial dilution. The results were analyzed with Gel-Pro-analyzer manager software

of them are not glycosylated. The PoIFN-*α* subtypes have very high homology and share 96-99.8% identity at the nucleotide level and 91.1-100% at the amino acid level [37]. Multi-sequence alignment revealed a C-terminal deletion of 8 residues in 6 subtypes. It was found that the antiviral activity of intact PoIFN-*α*s are 2-50 times higher than those subtypes with C-terminal deletions in WISH cells and 15-55 times higher in porcine kidney PK-15 cells. Interestingly, the highest degree of nucleotide divergence was found in the leader region of porcine IFN-*α* genes, which might include signals for intracellular storage of both dimers and monomers of some IFN-*α* subtypes during constitutive expression [38].

Comparative studies have showed that antiviral activity of porcine type I IFNs is virus- and cell-dependent. Sang et al.[39] reported that although most IFN-*α* subtypes retained the greatest antiviral activity against both *PRRSV* and *VSV* in porcine PK-15 cells and monkey MARC-145 cells, some IFNs including IFN-*α* 7/11 exhibited minimal or no antiviral activity in those target cell-virus systems. Also, Sosan et al.[40] found that most PoIFN subtypes *except* PoIFN-α5 and 7 showed excellent inhibition activity on the proliferation of classical swine fever virus. In the study performed by Cheng et al.[37], PoIFN expression was compared in 3 different systems including poly(I).poly(C)-DEAE-dextran induced PK-15 cells, pseudorabies virus infected PK-15 cells, and an attenuated strain of *swine fever virus* infected PK-15 cells. It was observed that expression of PoIFN-*α* was time-dependent in the former two systems, but was not such time-dependent in the third system.

So far, many IFN-α genes have been cloned and expressed in eukaryotic or prokaryotic cells [22,41,42]. Lefèvre et al.[22] expressed recombinant porcine IFN-α in the form of inclusion body in *E. coli* and the antiviral activity of refolded rPoIFNα was 6-fold greater than the natural porcine leukocyte interferon in the protection of porcine

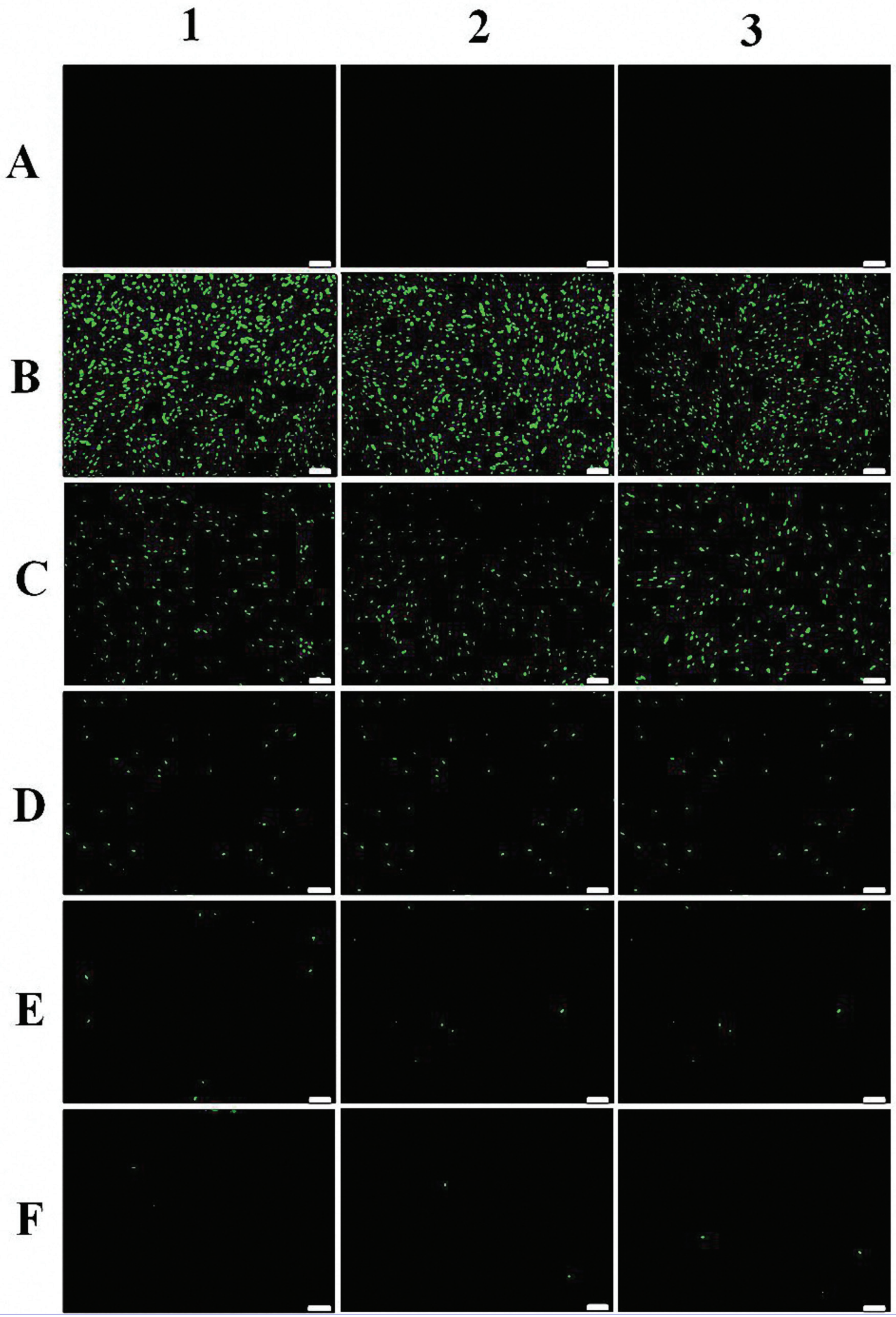

Fig 5. Immunofluorescence assay with three batches of rPoIFN-α on *TGEV* culture. 1: 2013001 batch of rPoIFN-α; 2: 2013001 batch of rPoIFN-α; 3: 2013001 batch of rPoIFN-α; A. cell control group. B. Virus control group. C. rPoIFN-α at 1:256 dilution. D: rPoIFN-α at 1:128 dilution. E. rPoIFN-α at 1:64 dilution. F. rPoIFN-α at 1:32 dilution

cells against *VSV* infection. Kim et al.[43] produced a recombinant mixture of *adenoviruses* bicistronically expressing porcine IFN-*α* and porcine IFN-γ and found it synergistically enhanced anti-*FMDV* effects compared with that of the *adenovirus* expressing a single IFN. More recently, a recombinant non-naturally occurring consensus porcine interferon-α (CoPoIFN-α) was designed by scanning 17 porcine IFN-α nonallelic subtypes and assigning the most frequently occurring amino acid in each position. It was revealed that the antiviral activity (units/mg) of CoPoIFN-α was higher than that of natural PoIFN-α in MDBK, PK-15 and MARC-145 cells [44]. In order to develop an IFN that might be used as an oral antiviral agent in animal health, PoIFN-α was successfully cloned and expressed in *Lactobacillus casei* with a vector that contains the inducible *lac* promoter and the secretion signal from an S-layer protein of *Lactobacillus brevis* [45].

Because the conventional production of interferon from natural leucocytes has disadvantages including low expression in healthy hosts and difficult extraction and purification procedures with high cost, large-scale preparation of rPoIFN-α with potent biological activities has become necessary. We achieved high level expression of the soluble form of bioactive rPoIFN-α in *E. coli* by selection of an appropriate expression vector pET32a. This vector contains Trx gene, which improves the solubility and activity of the rPoIFN-α protein [46,47]. The expression product of rPoIFN-α reached 32% of total bacterial proteins leading to the yields of 48 mg of recombinant PoIFN-α per liter of bacterial culture (data not shown). In addition, the His-tag carried by pET32a enables subsequent protein purification through Ni^{2+} affinity column. Our rPoIFN-α product was purified using essentially two-step chromatographic procedure which achieved biological activities as high as 1.1×10^6 IU/ ml. Furthermore, our rPoIFN-α is lyophilized and can be preserved at room temperature for a long period of time without carrier protein. The lyophilized product can be easily reconstituted in sterile saline or PBS. Therefore, comparing with native PoIFN-α , the rPoIFN-α we produced has many advantages in practical applications.

In summary, all the three batches of rPoIFN-α could inhibit the *TGEV*-induced cytopathic effect with consistent stable quality. The results of plaque formation assay, qRT-PCR, western blot and immunofluorescence assay showed that the rPoIFN-α had good inhibitory effect on the proliferation of *TGEV* in vitro. Thus, the current study suggested that the rPoIFN-α we produced has great potential for use as a novel antiviral agent in pig healthcare.

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