Effects from oral administration of recombinant interferon-alpha on piglet daily care

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Abstract

The administration of interferon has improved the antiviral and immunomodulatory abilities of piglets, which is conducive to the prevention of potential diseases or delay the appearance of clinical symptoms. This study aimed to evaluate the effects from administration of recombinant interferon-alpha (IFN- α) on the daily care of piglets. The results were compared with compound Chinese herbal, which was proved to improve serum interferon level. Further, the administration routes were compared between oral administration and intramuscular injection. Forty (40) piglets with equal age and weight were randomly divided into four groups: Control group (Group C, without treatment), Group H (treated with compound Chinese herbal), Group K (administered orally with recombinant IFN- α , 1500 IU per day per piglet), and Group J (administered intramuscularly with IFN- α , 4×10^6 IU per day per piglet). After the treatment of 15 days, both oral and intramuscular treatment of recombinant IFN- α significantly improved the secretion of IFN-gamma (IFN- γ) ($P < 0.05$), and the effects of intramuscular pathway were faster. In addition, the expression levels of IFN-stimulated genes (*MX1* and *ISG15*) were significantly enhanced ($P < 0.01$), independently of IFN- α treatment time and serum IFN- γ level. Different from other studies, compound Chinese herbal showed weaker effects on interferon stimulation in piglets. The results indicated that oral administration of recombinant IFN- α improved interferon-induced response of piglets at both serum and molecular levels, which may be applied for improving autoimmunity of piglets.

Introduction

Interferons (IFNs) have been known as a series of low molecular glycoproteins, which can interfere with viral infection and subsequent nucleic acid replication, thus suppressing viral proliferation. In addition, IFNs can also combat bacterial and parasitic infections. IFNs can be [categorized](#) into three distinct families according to its specific binding to the cell membrane receptors: type I (IFN- α and IFN- β), type II (IFN- γ) and type III (IFN- λ), and the endogenous IFNs are produced by leukocytes, fibroblasts and immune lymphocytes ([Musella, Manic, De Maria, Vitale, & Sistigu, 2017](#); [Renauld, 2003](#)). Attributed to their activities in immunoregulation, IFNs have been applied in both human health care and poultry industry ([Klotz, Baumgärtner, & Gerhauser, 2017](#)).

Exogenous administration of IFNs can improve the level of IFNs, and initiate a series of reactions and signal transduction by binding to the corresponding receptors on the cell membrane. The expression of hundreds of interferon-stimulated genes (ISGs) will be induced, which eventually suppress the infection of various RNA and DNA viruses ([Crosse, Monson, Beard, & Helbig, 2018](#)). IFN- α has been the mostly applied exogenous IFNs for clinical antiviral treatment, whose effects have been extensively proved ([Li et al., 2018](#)).

Recent studies demonstrated that the intramuscular injection of recombinant IFNs could be applied for the treatment of human influenza viruses ([Kroetz et al., 2015](#)), hepatitis viruses ([Hu, Ye, Ye, Wang, & Yu, 2019](#)), and antigen-induced arthritis ([Chalise, Narendra, Paudyal, & Magnusson, 2013](#)) etc. Similar results can also be obtained in animals, such as rhesus monkey ([Matzinger, Carroll, Fritts, McChesney, & Miller,](#)

2011), pig (Brockmeier et al., 2017; Liu et al., 2019; Yu et al., 2019), and broilers (Anjum, Rahman, Aslam, & Qureshi, 2020). The administration of IFN- α can inhibit the replication of porcine reproductive and respiratory syndrome virus (PRRSV) and enhance immune response in pigs (Yu et al., 2019). Further, after combining with inactivated influenza virus, IFN- α could alleviate clinical signs in pigs during the early viral infection (Liu et al., 2019). In short, IFN- α has been regarded as effective agents for the prevention and control of animal diseases.

The administration pathways of IFN- α have been investigated for years, including oral use (Cummins, Beilharz, & Krakowka, 1999; Tompkins, 1999), intramuscular injection, and nasal infusion as mucosal adjuvant (Liu et al., 2019). Different pathways exhibit various effects. The oral administration required only low dose for antiviral treatment, while intramuscular injection required high dose but significant immune responses. Nasal infusion of low-dose IFN- α was also effective in immune-stimulation. The administration pathways should be selected according to the specific applications.

Chinese herb is widely applied in the poultry industry, since several Chinese herb species have been proved to contain active compound for immune enhancement (Guo et al., 2012; C. M. Yang et al., 2019). The combination polysaccharide of *astragalus* polysaccharide and sulfated *epimedium* polysaccharide can significantly elevate serum HI antibody titers, IL-2 and IFN- γ levels (Guo et al., 2012). This study was conducted to evaluate the effects of recombinant IFN- α on immune performance in health piglets, with Chinese herb as comparison. The serum levels of IFN- γ and IFN-stimulated genes (*MX1* and *ISG15*) were evaluated as indicators. The administration pathways were compared between oral intake and intramuscular injection. This study proposed that oral administration of IFN- α may be a facile, practical and economic way for improving the immunity of piglets in daily care, and it may be also in conjunction with vaccine immunization. It may provide information for making up beneficial plans for health pig farms.

Materials And Methods

All animal care and handling were approved by the Ethics Committee for Animal Experimentation, science and technology department, Leshan Normal University, Sichuan, China. This trial was conformed to the health plan (Permission No.: WJSY2019) of pig farm of Beijing Vica biotechnology co., LTD., Beijing, China.

A total of 40 crossbred piglets (Landrace×Large White) with the same age (about 40 days old) and similar body weight were selected and labeled with ear number. All piglets were fed the same basal diet, which was formulated according to the nutrient requirements proposed by National Research Council (2012) (Table 1). Food and water were available *ad libitum*. All piglets weaned at 23 days of age were immunized with mycoplasma and porcine circovirus vaccine 7 days after weaning.

The compound Chinese herbal applied in this study was HLY provided by ShenYang Vica Animal Husbandry Technology Co., LTD., composed of astragalus, epimedium, fructus ligustris, etc. Each 1g of HLY is equivalent to 1.2g of crude drug by thin layer chromatography (TLC). Recombinant IFN- α was

provided by Beijing Vica Industrial Technology Innovation Research Institute. The titer of this product was detected as 3.89×10^9 U/mL with MDBK-VSV cell line. The endotoxin was tested as below 50 EU/ 10^7 U with gel clot test.

The 40 piglets were randomly divided into four groups (10 per group). The pigs in the same group were kept in one pen. The four groups were as follows: Group C (Control group without any treatment), Group H (treated with compound Chinese herbal 1:1000 diluted in water everyday), Group K (administered orally with recombinant IFN- α diluted in distilled water every day, 1500 IU per day per piglet), and Group J (administered intramuscularly with recombinant IFN- α diluted in saline every day, 4×10^6 IU per day per piglet). The treatment was lasted for 15 days.

Table 1 Basic diet formula and nutrition level

Ingredients	Proportion (%)	Nutrition index **	Nutritional content
Corn	63	CP	17.50%
46% Soybean meal	10	Lys	1.35%
Puffed soybeans	7.5	Ca	0.62%
Fermented soybean meal	5	NE (Mcal)	2600
Steam fishmeal	2.5		
Whey powder	4		
sucrose	2		
Soybean oil	2		
Premix *	4		

Note: * Premix provided multi-dimensional and multi-mineral, salt, acidulant, phytase, pharmaceutical additives, etc. ** Nutritional index was the calculated value.

The whole blood was collected from the precaval vein of piglets at 8:00 a.m. on Days 1, 2, 3, 6, 9, 12, and 15. The blood samples with and without anticoagulation (EDTA) were obtained. Serum was separated from non-anticoagulated blood and stored frozen at -20°C for test. The peripheral blood lymphocytes (PBMC) were separated from anticoagulated blood. The total RNA of PBMC was extracted with the *TRIZOL* reagent (Invitrogen) and purified with DNase according to the manufacture’s manual. The RNA had an OD_{260nm}: OD_{280nm} ratio between 1.8 and 2.0. The RNA was reverse-transcribed, and stored frozen at -80 ° C for following tests.

The IFN- γ level in serum samples was detected by American Cygnus mouse γ -interferon ELISA kit, according to the manufacture’s manual. The r^2 value of standard curve was 99.2%, and the IFN- γ level was calculated according to the standard curve.

Primers were designed according to the gene sequences of *MX1* and *ISG15* in pigs and the primers of internal reference gene *β -actin* were published in NCBI (Table 2). The real-time PCR was performed with the cDNA template previously transcribed from extracted RNA. Real-time PCR was performed to detect the expression levels of *MX1* and *ISG15* with the SuperReal PreMix Plus (SYBR

Green) kit (Tiangen, FP205). The optimized PCR conditions were: 95 °C for 15 min; 95 °C for 10 s, 60 °C for 20 s, 72 °C for 20 s, 40 cycles; 95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s to collect the fluorescence signals. Each sample was repeatedly tested three times. The PCR products were ligated into pGM-Simple-T vector and transformed into *E. coli* DH5α to obtain the recombinant plasmids pTpo*MX1*, pTpo/*SG15* and pTpoβ-actin. After being identified and verified by PCR and sequence, these plasmids were stored at -80 °C.

The relative mRNA expression was calculated by $2^{-\Delta Ct}$ (ΔCt = Ct of the target gene-Ct of the housekeeping gene), and β-actin was taken as internal reference gene in this study. Real-time PCR efficiency was acquired by the amplification of serial dilution of plasmids contained target fragment according to the equation $10^{(-1/\text{slope})}$ and kept consistent between target genes and β-actin. Negative controls were performed in which water was substituted for cDNA.

Table 2 Primer sequence for IFN stimulation genes and internal reference gene

Gene	NCBI ref.	Primer	Sequence (5' to 3')	PCR Length
β-actin	U07786	β-actin F1	CTTCCTGGGCATGGAGTCC	201 bp
		β-actin R1	GGCGCGATGATCTTGATCTTC	
<i>MX1</i>	M65087	<i>MX1</i> F1	CATCTGTAAAACTCTGCCCCTGT	115 bp
		<i>MX1</i> R1	CATCTTCCCGCTTTCATCCT	
<i>ISG15</i>	EU584557.1	<i>ISG15</i> F2	TGGTGAGGAACGACAAGGGTC	128 bp
		<i>ISG15</i> R2	CTCGAAAGTCAGCCAGAAATGGT	

Statistical analysis was performed using SPSS17.0. One-way ANOVA and LSD method was used to analyze the significance of differences among groups. The general linear model GLM program was used to analyze the significance of the two factors (treatment time and administration pathways) on the evaluation indexes. Pearson correlation coefficient (PCC) of bilateral test was used to analyze the correlation between IFN-γ and IFN-stimulated genes. $P<0.05$ indicated significant difference, and $P<0.01$ indicated extremely significant difference. The test data were presented as mean ± standard error.

Results And Discussion

Effects from different treatments on IFN-γ level in piglet serums were shown (Figure 1). The administration of IFN-α showed significant enhancement of serum IFN-γ compared to that of Chinese herb and control. On Day 1, 2 and 3 of treatment, the level of serum IFN-γ in piglets of Group J was significantly higher than that of other groups ($P<0.05$). Compared to Group C, the IFN-γ level in Group J was increased by 118% and 84% on Day 1 and Day 2, respectively. On Day 6, 9, 12 and 15, the IFN-γ level of Group K was gradually increased, which was much higher than that of other groups. Compared to Group H, the IFN-γ level in Group K was significantly increased by 231% and 308% on Day 6 and Day 9, respectively ($P<0.05$). On Day 15, the IFN-γ level in Group K was increased by 345%, 531%, and 173%, respectively, compared to that of in Group C, Group H, and Group J ($P<0.05$). The intramuscular injection

of IFN- α showed significant increase of serum IFN- γ at the first 3 days, and oral administration showed gradual increase of serum IFN- γ during 6-15 days after the treatment.

Effects from different treatments on the expression level of *MX1* mRNA in PBMC of piglets were shown (Fig. 2). The administration of IFN- α elevated the level of *MX1* mRNA compared to that of Chinese herb and control. On Day 9, the *MX1* mRNA in piglets of Group J was significantly increased by 64% than that of Group C ($P < 0.05$), while no significant difference was observed at other treatment time points. The *MX1* mRNA in piglets of Group K was increased by 89% compared to that of Group H on Day 2 ($P < 0.05$), and it was increased by 47% than that of Group J on Day 3 ($P < 0.05$).

Effects from different treatments on the expression level of *ISG15* mRNA in PBMC of piglets were shown (Figure3). Both the administration of IFN- α and Chinese herb elevated the level of *ISG15* mRNA. On Day 3, the level of *ISG15* mRNA in Group K and Group H was increased by 13% and 28%, respectively, compared to that of Group C ($P < 0.05$). No significant difference was observed in other treatment time points.

Correlation between treatments and evaluation indexes was explored in all samples, including serum IFN- γ , expression level of *MX1* and *ISG15* mRNA. The different treatments had significant effects on serum IFN- γ and relative mRNA expression abundance of *MX1* and *ISG15* ($P < 0.01$). However, the treatment duration had no significant effects ($P > 0.05$).

Correlation between serum level of IFN- γ and the expression level of IFN-stimulated genes in PBMC was explored in all samples ($n = 280$, table 3). No significant correlation was observed between serum IFN- γ with *MX1* or *ISG15* mRNA level in PBMC ($P > 0.05$). Good correlation was observed between the expression of *MX1* and *ISG15* mRNA, with PCC of 0.771. The result was extremely significant ($P < 0.01$).

Table 3. Correlation of serum IFN- γ and IFN stimulated genes in PBMC

Index	serum IFN- γ	<i>MX1</i> mRNA	<i>ISG15</i> mRNA
serum IFN- γ	1	0.006	-0.01
<i>MX1</i> mRNA		1	0.771**
<i>ISG15</i> mRNA			1

Note: ** Indicated extreme significance, $P < 0.01$.

The supplementation of IFN- α has been widely applied in livestock and poultry industry. In recent years, the effects of IFN- α have been investigated, as immunoregulator, antivirals, vaccine adjuvant and immunopotentiator etc. (Li et al., 2018) The oral administration of IFN- α in pig showed better adaptive immune response (Brockmeier et al., 2017; Razzuoli, Dotti, Archetti, & Amadori, 2010). It was resulted from the regulatory effect on IFN- γ gene and the increase of IFN- γ -secreting lymphocytes. Thus, the serum IFN- γ can be applied as an indicator for the effects of IFN- α (Razzuoli et al., 2010). Further, antiviral activity of IFN- α was also reported. The recombinant, replication-defective human adenovirus type 5

vectors containing porcine IFN- α (Ad5-pIFN α) was constructed. The pigs inoculated with 10^9 PFU of Ad5-pIFN α were completely protected when challenged 24 h later with foot-and-mouth disease virus (FMDV). The animals showed no clinical signs, no viremia, and did not develop antibodies against viral nonstructural proteins, achieving complete protection from FMDV infection (Chinsangaram, Moraes, Koster, & Grubman, 2003; Moraes et al., 2007). After the administration of a nonreplicating human adenovirus type 5 vector expressing porcine IFN- α , innate and adaptive immune responses of pigs to PRRSV can be improved. Viremia was delayed, and viral load was decreased in the sera of pigs. Further, the number of virus-specific IFN- γ -secreting cells was increased (Brockmeier et al., 2012). In another study, IFN- α was administered via an adenovirus vector as an adjuvant with live-attenuated PRRSV vaccine for enhancing immune response to the vaccine. However, the assumed adjuvant effect was not observed and IFN- α inhibited replication of the vaccine virus (Brockmeier et al., 2017). IFN- α was also applied as mucosal adjuvant for influenza vaccine in pigs. The combination of low-dose IFN- α and inactivated influenza virus via nasal infusion could significantly up-regulate the expression of immunoregulatory cytokines and induce a strong mucosal innate immune response (Liu et al., 2019).

Most of previous studies focused on the effects of IFN- α against virus, in our study, the health piglets were administrated with IFN- α . The effects on serum IFN- γ were explored. The results proved that the IFN- α was able to elevate the serum IFN- γ level, as well as the expression levels of IFN-stimulated genes, in the subsequent days after the treatment. The result may help in controlling viral diseases during daily care of piglets, especially for the farms at the threaten of virus infection.

The administration pathways have been another important factor for affecting the effects of IFN- α , which should be selected according to the specific applications. One study compared the therapeutic effect of natural chicken IFN- α administered via oral and intramuscular (i.m.) routes against Newcastle Disease (ND) in broiler chicken. The protection effects were better in chicken treated with IFN- α via the oral route than in those treated via the i.m. route (Anjum et al., 2020). Similarly, broilers were administrated with recombinant IFN- α via intravenous, intramuscular, and subcutaneous injections. The results showed that the half-life of IFN was faster, reaching a peak in about 3~4 hours (Zhao et al., 2017). In previous study, after the intramuscular injection of 24.5×10^6 IU IFN- α 2b and IFN- γ complex, serum IFN- α and IFN- γ began to rise at several hours after the treatment, and then declined after reaching the peak in the human body (García-García et al., 2016). In our study, the oral administration (1500 IU per day per piglet) and intramuscular injection (4×10^6 IU per day per piglet) was compared. The oral administration exhibited a gradually increased efficacy lasted for 10 days, while the intramuscular injection presented a rapid but quickly weakened efficacy lasted for only a few days. The oral administration was economic and effective, in addition, much facile than that of other pathways, such as the construction and injection of IFN- α loading vectors.

The active ingredients in traditional Chinese herb have been reported to regulate the IFN secretion. Studies found that the content of serum IFN- γ was significantly increased after 21 days of applying astragalus polysaccharides in piglet diets, but it had no significant effect on IgG, IL-4 and IL-10 (Yuan et al., 2006). A similar result found that a compound traditional Chinese herb based on *astragalus*

polysaccharide, *epimedium* polysaccharide, *propolis flavonoids* and *saponins* significantly increased the mRNA relative expression levels of IFN- γ and IL-10 (L. Yang et al., 2008). However, in our study, it found that compound traditional Chinese herb, involving *astragalus*, *epimedium*, *privet*, etc., made no significant effect on piglet serum IFN- γ . The reason may be the improper compatibility, insufficient amount, or slow efficacy. Compared to traditional Chinese herb, the administration of IFN- α may provide a more controllable efficacy.

Antiviral proteins MX1 and ISG15 are secreted by JAK/STAT signaling in an unconventional secretion pathway (Novakova et al., 2010; Toyokawa, Carling, & Ott, 2007). In this study, the relative mRNA expression levels of IFN-stimulated genes *MX1* and *ISG15* were significantly correlated, indicating that *MX1* and *ISG15* were expression-related genes. The determination of *MX1* and *ISG15* may assist in exploring the mechanism of IFN-stimulated responses. From the results of our study, it found that the treatment method significantly affected the mRNA expression levels of *MX1* and *ISG15*. For *MX1*, the oral administration of recombinant IFN- α can significantly elevate its mRNA expression level on Day 2 of treatment, while the intramuscular administration can significantly increase the mRNA expression level on Day 9. For *ISG15*, the oral administration of recombinant IFN- α can increase its mRNA expression level on Day 3. No significant difference was observed in other treatment time points. Further analysis revealed no correlation between serum IFN- γ and IFN-stimulated genes. It indicated that exogenous treatment can regulate the serum IFN- γ and IFN-stimulated genes independently. Due to the complexity of immune-regulation, the mechanism of IFN- α treatment remained to be explored.

Conclusion

The administration of IFN- α made positive effects on the antiviral and immunomodulatory abilities of piglets, exhibited as improved serum level of IFN- γ and mRNA of IFN-stimulated genes (*MX1* and *ISG15*) in PBMC. Compared to that of the intramuscular administration, the oral pathway presented gradually elevated level of serum IFN- γ and lasted for 15 days. It provided an economic and facile pathway to improve the autoimmunity of piglets, which can be applied in daily care in swine farm.

Declarations

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Authors' contributions

FD and BZ initiated the idea, finished the experiment design. FD, YL, MZ and RG conducted the animal trial and laboratory analysis. TL and HC prepared the initial manuscript in English. FD made the final

revision of the manuscript. All authors read and approved the final manuscript.

Conflict of interest declaration

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work. All results were strictly in accordance with the experimental data.

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Figures

Figure 1

Effects from different treatments on serum IFN- γ level in piglets

^{a,b} Groups identified with a similar letter above the bar at a time point were not different with probability $P = 0.05$

Figure 2

Effects from different treatments on the expression level of *MX1* mRNA in PBMC

^{a,b} Groups identified with a similar letter above the bar at a time point were not different with probability $P = 0.05$)

Figure 3

Effects from different treatments on the expression level of *ISG15* mRNA in PBMC