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Original article

Pharmacokinetics of the recombinant ovine interferon-tau in lambs

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Abstract

In the current study, twenty lambs, aged 4 months, half male and half female, were classified into four groups, with five in each group. The experimental three groups of lambs were given intravenous (IV), intramuscular (IM) and subcutaneous (SC) administrations of recombinant ovine interferon-τ (roIFN-τ). The fourth group (normal control) of lambs was given normal saline injections in the same way. After administrations, blood samples were collected from the tested animals at different time points post injection, and the serum titers of roIFN-τ were measured using cytopathic effect (CPE) inhibition bioassay. The results of calculating pharmacokinetic (PK) parameters using DAS software showed that the PK characteristics of roIFN-τ through IV injection conformed to the two-compartment open model, whose half-life of distribution phases ($T_{1/2\alpha}$) was 0.33±0.034 h and the elimination half-life($T_{1/2\beta}$) was 5.01±0.24 h. However, the PK features of IM injection and SC injection of roIFN-τ conformed to the one compartment open model, whose T_{max} were 3.11±0.26 h and 4.83±0.43 h, respectively, together with an elimination half life($T_{1/2\beta}$) of 9.11±0.76 h and 7. 43±0.58 h, and an absorption half-life ($T_{1/2k(a)}$) of 1.13±0.31 h and 1.85±0.40 h, respectively. The bioavailability of roIFN-τ after IM administration reaches 73.57%, which is greater than that of SC administration (53.43%). These results indicate that the drug administration effect can be preferably obtained following a single dose IM administration of the roIFN-τ aqueous preparation. This study will facilitate the clinical application of roIFN-τ as a potential antiviral agent in future work.

Key words: bioavailability, ovine interferon-τ, cytopathic effect inhibition assay, pharmacokinetic study

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Introduction

Interferons (IFNs) are a large protein family with antiviral, immunomodulatory, and cell growth regulatory activities (Bonjardim 2005, Tian et al. 2014). It was first reported by Isaacs and Lindenmann in 1957 that influenza-virus-infected chicken cells could produce a soluble factor exerting resistance to homologous and heterologous viruses (Isaacs and Lindenmann 1957). Interferon-tau (IFN-τ) has a wide spectrum of antiviral activities against a variety of human and animal viruses and belongs to the first of three categories of IFN (Chon and Bixler 2010). Its amino acids homologize with the IFN-α and IFN-ω are 55% and 70%, which allow it to bind to the type I IFN receptor and activate the JAK-stat pathway, eventually leading to the generation of antiviral proteins such as PKR, ADARI, OAS, and Mx proteins (Samuel 2001). Ovine IFN-τ is not induced by virus, but has been proved to have antiviral activity against human papilloma virus (Johnson et al. 1999), Human Immunodeficiency Virus (Pontzer et al. 1997), Feline Immunodeficiency Virus (Pontzer et al. 1997), ovine lentivirus (Juste et al. 2000), and foot-and-mouth disease virus (Usharani et al. 2017). Additionally, IFN-τ has a much lower cytotoxicity at higher concentrations of use in comparison to IFN-α (Soos et al. 1995, Usharani et al. 2017). These attributes provide a solid foundation for its future clinical applications.

IFN has shown species specificity. As a result, research on the toxicology, PKs and general pharmacology of Ovine IFN-τ must be carried out in lambs and cannot be studied in human or other animals (Einhorn and Strander 1977). It is necessary to properly evaluate the PK characteristics of ovine IFN-τ before its use in clinical practice. However, to the best of our knowledge, no report on the PK of roIFN-τ in lambs is so far available. Therefore, in this study, we aimed to investigate the PK behavior of roIFN-τ, following the administration of a single injection intravenously or subcutaneously or intramuscularly, by assessing the serum roIFN-τ biological activities at a variety of time points post injection using the cytopathic effect (CPE) inhibition bioassay.

Materials and Methods

Animals and Reagents

In this study, twenty lambs (*Ovis arie*) aged 4 months were used, including 10 males and 10 females. All lambs were purchased from a commercial sheep farm at three months of age, and were fed *ad libitum* with commercial diet for a month in the Experimental Animal Research Center of Anhui Province (Hefei, Anhui, P.R.China). The lambs weighed from 16.9 kg to 24.4 kg (20.2±4.3 kg), and they were randomly classified into 4 groups, with 5 lambs per group.

The roIFN-τ freeze-dried powder for animal injection was offered by the Anhui Jiuchuan Biotech Co, Ltd. (Batch number: 20150608, Wuhu, Anhui, P.R.China), and was made in the yeast (*Pichia pastoris*) expression system using molecular biology techniques as previously described (Juste et al. 2000). The roIFN-τ was obtained following the procedure of purification, sterilization and lyophilization. The titer of roIFN-τ was 1.0×10^8IU /vial.

Within these four lambs groups, the first group was injected with roIFN-τ intravenously, at a dose of 8.0×10^7IU/kg. The second group and the third group were given IM injection and SC injection of roIFN-τ at the same dose, respectively. The fourth group (Normal Control lambs) was given normal saline injection using the same methods.

All procedures performed in studies involving animals were in accordance with the ethical standards of the Research Ethics Committee of Anhui Medical University for animal experiments.

Sample collection

Following roIFN-τ or saline treatment, blood samples were collected from the cervical vein in all groups of animals at 0, 0.25, 0.50, 1, 2, 3, 4, 6, 8, 12, 24, and 48 h. The resulting blood liquids were then incubated at room temperature for 2 hours to coagulate. They were then centrifuged at 3000×*g* at 4°C for 10 minutes. Finally, the supernatants (sera) were stored at -70°C until use. The animal experimental protocol performed in this study was approved by the Institutional Ethics Committee of Anhui Medical University (approval number: LLSC20170364). No safety problems were observed at this dosing level during or after roIFN-τ treatment in this study.

RoIFN-τ analysis in serum

The titer of roIFN-τ was measured through the challenge of Madin–Darby bovine kidney epithelial cells (MDBK) by vesicular stomatitis virus (VSV) using the CPE inhibition assay as described previously (Juste et al. 2000). In brief, the MDBK cells were inoculated into the 96-well micro-test plates at a density of 3×10^4 cells per well and incubated in DMEM containing 3% fetal calf serum (FCS) at 37°C and 5% CO_2 humid air for 12 h. The monolayers of MDBK cells were treated with 100 μl of 4-fold serial diluted roIFN-τ liquid. After 24-h incubation, the cells were attacked by VSV at a volume of 100 $TCID_{50}$/well (50% tissue culture infection dose) and continued to be cultured until the

Table 1. Main pharmacokinetic parameters for roIFN-τ for injections in both male and female lambs (n=5, $\bar{x}$±SD).

Parameter	IV injection group	IM injection group	SC injection group
T_{max}(h)	–	3.11±0.26	4.83±0.43#
C_{max}(×10^5IU/mL)	–	8.25±0.44	6.73±0.35#
$AUC_{(0-t)}$(IU /mL*h)	1254271.47±92643.25	918125.37±56842.15*	683555.68±48941.34*#
$AUC_{(0-\infty)}$ (IU /mL*h)	1261224.62±87566.41	927921.25±44285.32*	673916.49±48436.27*#
Cl(mL/h)	1281.29±83.18		
Cl/F(mL/h)		412.29±19.41	340.95±25.27
MRT(h)	6.27±0.56	15.84±1.63*	14.22±1.23*
$V_{d(ss)}$(mL)	1747.32±124.57		
V_d/F (mL)		526.25±78.67	372.60±36.33#
F		73.57%	53.43%#
$T_{1/2\alpha}$(h)	0.33±0.03	-	-
$T_{1/2\beta}$(h)	5.01±0.24	9.11±0.76*	7. 43±0.58*
$T_{1/2k(a)}$	-	1.13±0.31	1.85±0.40#
k_{10}(1/h)	3.96±0.62		
k_{12}(1/h)	0.19±0.02		
k_{21}(1/h)	0.14±0.01		

Data are expressed as mean±SD (n=5); * $p<0.05$ compared with the IV group, # $p<0.05$ compared with the IM group; T_{max}: time of maximum concentration observed in serum; C_{max}: maximum drug concentration; $T_{1/2k(a)}$: absorption half-life; $T_{1/2\alpha}$: half-life of distribution phases; $T_{1/2\beta}$: elimination half life; $AUC_{(0-\infty)}$: area under the concentration time-curves from time zero to infinity; $AUC_{(0-t)}$: area under the concentration time-curves from administration to last observed concentration at t; $V_{d\ (ss)}$: volume of distribution at steady state; V_d/F: apparent volume of distribution; K_{12}: rate of transfer of drug from central to peripheral compartment; K_{21}: rate of transfer of drug from peripheral to central compartment; $K_{10:}$ elimination rate constant; Cl: total body clearance; Cl/F: apparent clearance; MRT: mean residence time; F: bioavailability.

appearance of 100% CPE in the virus-infected cells (virus control well without roIFN-τ treatment). Prior to plaque counting, the culture was stained with crystal violet. One IFN unit was defined as the highest dilution of roIFN-τ that inhibited 50% CPE when 100% CPE was found in the non-IFN treated control wells. The titers (IU) of roIFN-τ were calculated as the reciprocal of the dilutions resulting in 50% cell lysis using the Reed-Muench method (Reed and Muench 1938). We adopted a recombinant human IFN-α (rhIFN-α1, 3×10^6 IU/ml, Lot number 97/04), which was taken from the China Food and Drug Inspection Institute (Beijing, P.R.China) as a positive control for this bioassay. The precision of the IFN standard, expressed in %RSD, was 2.71%. The accuracy of the IFN standard, expressed in relative mean error (RME), was less than 10.12%. In the current study, both the precision and accuracy values have met the requirements.

Statistical analysis and data processing

Numerical variables are presented as the arithmetic mean±standard deviation ($\bar{x}$±SD). The data for serum roIFN-τ concentrations at all time points following IV, IM and SC administrative injections were computed using the curve fitting formula using DAS (Drug and statistics) software (Version 2.0, Wenzhou Medical University, Wenzhou, Zhejiang, China) (Chen et al. 2015). The standard PK parameters included absorption half-life ($T_{1/2k(a)}$), half-life of distribution phases ($T_{1/2\alpha}$), elimination half life ($T_{1/2\beta}$), area under the concentration time-curves from time zero to infinity ($AUC_{(0-\infty)}$), area under the concentration time-curves from administration to last observed concentration at t ($AUC_{(0-t)}$), volume of distribution at steady state ($V_{d\ (ss)}$), Apparent Volume of Distribution (V_d/F), rate of transfer of drug from central to peripheral compartment (K_{12}), rate of transfer of drug from peripheral to central compartment (K_{21}), elimination rate constant (K_{10}), clearance rate (Cl), apparent clearance (Cl/F), maximum drug concentration (C_{max}), time of maximum concentration observed in serum (T_{max}), bioavailability (F), and mean residence time (MRT). These serum concentration data were standardized with the animal's body weight by a comparative analysis. The AUC values after SC administration were computed through the linear-up/log-down trapezium method. To calculate $AUC_{0-\infty}$ and Cl, a terminal rate was measured with

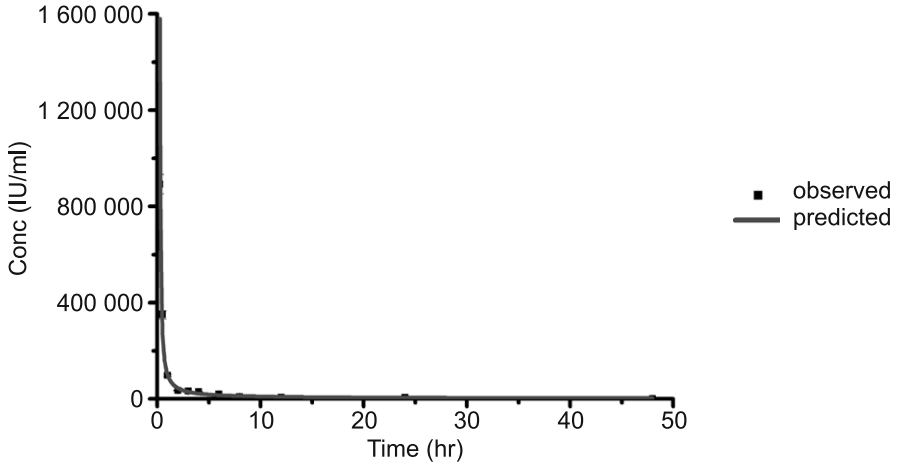

Fig. 1. Serum IFN titer-time curves for the roIFN-τ IV injection group
Serum IFN titer-time curve in the roIFN-τ IV administration group, where the X-axis represents time and the Y-axis stands for the titer of roIFN-τ. The scattered squares indicate the average value, while the Y error bars indicate standard deviations. The IV injection group was tested 3 times.

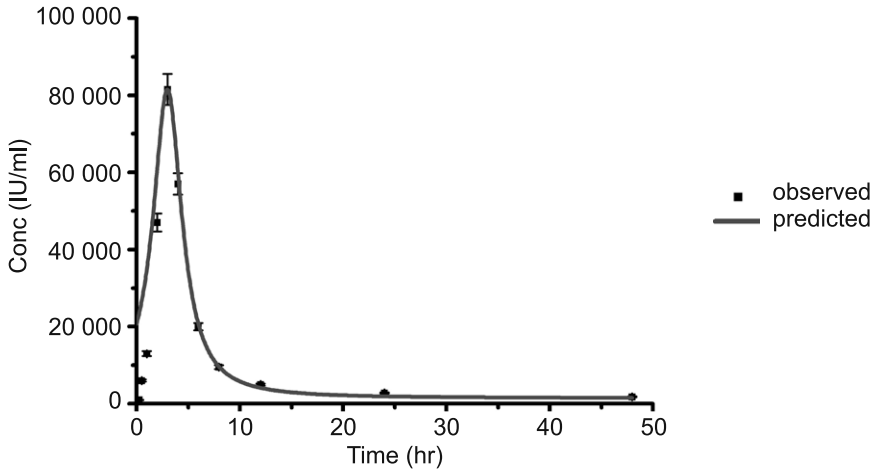

Fig. 2. Serum IFN titer-time curves for the roIFN-τ IM injection group
Serum IFN titer-time curve in the roIFN-τ IM administration group, where the X-axis represents time and the Y-axis stands for the titer of roIFN-τ. The scattered squares indicate the average value, while the Y error bars indicate standard deviations. The IM injection group was tested 3 times.

the slope curve extended to 48 h. Moreover, unpaired Student's t-test was used to compare the data of the anti-viral activity in sera collected from the group of roIFN-τ-treated animals with the group of normal-saline-treated control animals in each day. The significance level (α) was set at 0.05.

The data were plotted using OriginLab software version 8.5 (OriginLab, Northampton, MA, USA); the predicted curve of IFN concentration was fitted using the nonlinear fitting method of this software. The formula for bioavailability calculation was according to the following equation:

$$F = \frac{AUCs.c.\text{ or } i.m. \times Di.v.}{AUCi.v. \times Ds.c.\text{ or } i.m.} \times 100\%.$$

(F: Bioavailability; AUCs.c. or i.m.: $AUC_{(0-\infty)}$ for SC administration or $AUC_{(0-\infty)}$ for IM administration; Di.v.:Dose for IV administration; AUCi.v.: $AUC_{(0-\infty)}$ for IV administration; Ds.c. or i.m.: Dose for SC administration or Dose for IM administration).

Results

The experimental lambs were given IV, IM or SC injection of roIFN-τ at a dose of 8.0×10^7IU/kg, and the blood roIFN-τ efficacy was measured using the calculation of its anti-viral activity in the MDBK cell infected by VSV.

The PK parameters of roIFN-τ determined by three different administration routes in tested lambs are shown in Table 1. The PK characteristics of roIFN-τ in the group of IV injection conformed to the two-compartment open model, which was associated with first-order elimination. For IV administration, the $T_{1/2\alpha}$ was 0.331±0.034 h and the $T_{1/2\beta}$ was 5.011±0.24 h (Fig. 1).

Table 2. Main pharmacokinetic parameters for roIFN-τ for administrations in male lambs ($\bar{x}$±SD).

Parameter	IV injection group	IM injection group	SC injection group
T_{max}(h)	-	3.12±0.26	4.84±0.43#
C_{max}(×10^5IU/mL)	-	8.26±0.44	6.74±0.35#
$AUC_{(0-t)}$(IU /mL*h)	1254373.47±92645.25	918135.37±56843.15*	683565.68±48942.34*#
$AUC_{(0-\infty)}$ (IU /mL*h)	1261334.62±87567.41	927931.25±44286.32*	673926.49±48438.27*#
Cl(mL/h)	1281.49±83.18		
Cl/F(mL/h)		412.31±19.41	340.96±25.27
MRT(h)	6.27±0.56	15.84±1.63*	14.22±1.23*
$V_{d(ss)}$(mL)	1747.52±124.57		
V_d/F (mL)		526.26±78.67	372.62±36.33#
F		73.58%	53.44%#
$T_{1/2\alpha}$(h)	0.33±0.03	-	-
$T_{1/2\beta}$(h)	5.01±0.24	9.11±0.76*	7. 43±0.58*
$T_{1/2k(a)}$	-	1.13±0.31	1.85±0.40#
k_{10}(1/h)	3.96±0.62		
k_{12}(1/h)	0.19±0.02		
k_{21}(1/h)	0.14±0.01		

Data are expressed as mean±SD; * $p<0.05$ compared with the IV group, # $p<0.05$ compared with the IM group; T_{max}: time of maximum concentration observed in serum; C_{max}: maximum drug concentration; $T_{1/2k(a)}$: absorption half-life; $T_{1/2\alpha}$: half-life of distribution phases; $T_{1/2\beta}$: elimination half life; $AUC_{(0-\infty)}$: area under the concentration time-curves from time zero to infinity; $AUC_{(0-t)}$: area under the concentration time-curves from administration to last observed concentration at t; $V_{d\ (ss)}$: volume of distribution at steady state; V_d/F: apparent volume of distribution; K_{12}: rate of transfer of drug from central to peripheral compartment; K_{21}: rate of transfer of drug from peripheral to central compartment; $K_{10:}$ elimination rate constant; Cl: total body clearance; Cl/F: apparent clearance; MRT: mean residence time; F: bioavailability.

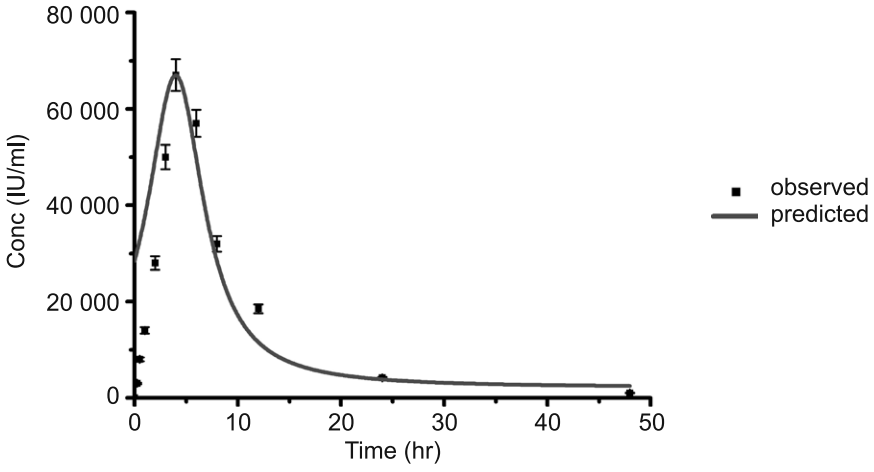

Fig. 3. Serum IFN titer-time curves for the roIFN-τ SC injection group
Serum IFN titer-time curve in the roIFN-τ SC administration group, where the X-axis represents time and the Y-axis stands for the titer of roIFN-τ. The scattered squares indicate the average value, while the Y error bars indicate standard deviations. The SC injection group was tested 3 times.

The PK characteristics of roIFN-τ in the groups of IM injection and SC injection conformed to the one compartment open model. Their T_{max} was 3.11±0.26 h and 4.83±0.43 h, respectively. Their $T_{1/2\beta}$ were 9.11±0.76 h and 7.43±0.58 h, their $T_{1/2k(a)}$ were 1.13±0.31 h and 1.85±0.40 h, respectively (Figs. 2 and 3).

The bioavailability of roIFN-τ in the IM administration group was 73.57%, and the bioavaila-

Table 3. Main pharmacokinetic parameters for roIFN-τ for administrations in female lambs ($\bar{x}$±SD).

Parameter	IV injection group	IM injection group	SC injection group
T_{max}(h)	–	3.10±0.26	4.82±0.43#
C_{max}(×10^5IU/mL)	–	8.24±0.44	6.72±0.35#
$AUC_{(0-t)}$(IU /mL*h)	1254171.48±92642.31	918115.34±56841.12*	683545.68±48940.24*#
$AUC_{(0-\infty)}$ (IU /mL*h)	1261122.60±87565.40	927910.22±44285.32*	673905.46±48435.18*#
Cl(mL/h)	1281.19±83.18		
Cl/F(mL/h)		412.28±19.41	340.94±25.26
MRT(h)	6.27±0.56	15.84±1.63*	14.22±1.23*
$V_{d(ss)}$(mL)	1747.22±124.56		
V_d/F (mL)		526.24±78.66	372.59±36.32#
F		73.56%	53.42%#
$T_{1/2\alpha}$(h)	0.33±0.03	–	–
$T_{1/2\beta}$(h)	5.01±0.24	9.11±0.76*	7. 43±0.58*
$T_{1/2k(a)}$	–	1.13±0.31	1.85±0.40#
k_{10}(1/h)	3.96±0.62		
k_{12}(1/h)	0.19±0.02		
k_{21}(1/h)	0.14±0.01		

Data are expressed as mean±SD ; * $p<0.05$ compared with the IV group, # $p<0.05$ compared with the IM group; T_{max}: time of maximum concentration observed in serum; C_{max}: maximum drug concentration; $T_{1/2k(a)}$: absorption half-life; $T_{1/2\alpha}$: half-life of distribution phases; $T_{1/2\beta}$: elimination half life; $AUC_{(0-\infty)}$: area under the concentration time-curves from time zero to infinity; $AUC_{(0-t)}$: area under the concentration time-curves from administration to last observed concentration at t ; $V_{d\ (ss)}$: volume of distribution at steady state; V_d/F: apparent volume of distribution; K_{12}: rate of transfer of drug from central to peripheral compartment; K_{21}: rate of transfer of drug from peripheral to central compartment; $K_{10:}$ elimination rate constant; Cl: total body clearance; Cl/F: apparent clearance; MRT: mean residence time; F: bioavailability.

bility of roIFN-τ in the SC administration group was 53.43%.

Regarding gender difference, the PK parameters did not differ significantly between sexes at this dosing level (Table 2 and 3).

Discussion

In the last several years, there has been a significant increase in the number of peptides and protein drugs available for clinical use (Kumar et al. 2006). Advances in science and technology in recent decades have provided an increasing number of tools and opportunities for the development of peptides and proteins as drugs. For treatment, most peptide and protein drugs are usually applied through invasive pathways such as IV, SC or IM injections, due to the low bioavailability and high enzymatic degradation rates in the stomach and small intestine (Yamazaki et al. 2010). Additionally, some supportive evidence indicates that the route of administration, namely, the behavior of drug treatments, is a significant factor affecting the efficacy of treatment (Wills 1990). Therefore, in this study, we chose IV, SC and IM injection methods to investigate the PK profiles of roIFN-τ in lambs.

The PK characteristics of human IFN have been fully described (Wills 1990). Its blood concentration is rapidly decreased soon after IV administration, and the distribution volume is close to 20-60% of body weight (Wills 1990). Animal study suggests that the catabolism type of IFN belongs to the category of the natural processing of proteins (Wills 1990). The Clearance value of the entire IFN family varies from one to another (range: 4.8-206 L/h) (Zhao et al. 2017), which may reflect the natural digestion and the regeneration of proteins. The terminal elimination half-life of IFN-α is from 4-16 hours (Wills 1990). However, in the current study, we detected a Cl of 1281.29±83.18 (mL/h), a $Vd_{(ss)}$ of 1747.32±124.57 (mL) and a $T_{1/2\beta}$ of 5.01±0.24 (h) through IV administration of roIFN-τ. Therefore, the PK parameters of roIFN-τ, such as Vd and Cl, measured in this study, are generally lower than those of human IFN-α.

The PK features of IM injection and SC injection of roIFN-τ conformed to the one compartment open

model, their elimination half-life ($T_{1/2\beta}$) is 8.35-9.87 h and 6.78-7.72 h, respectively. These values are similar to those of human IFN-α (Osborn et al. 2002).

The absorption rate of roIFN-τ by IM injection was faster than that by SC injection. The AUC value in the SC injection group was about 2/3 of that in the IM injection group. These results showed that, with the same dosage, the peak value of the drug in the blood circulation in the SC group was lower than that in the IM group, and the drug intake in the SC group was also lower than that in the IM group. Therefore, SC administration of roIFN-τ could result in a relatively low level of concentration maintained in the blood circulation.

The bioavailability of roIFN-τ in the IM group is 73.57%, and the bioavailability of roIFN-τ in the SC group n is 53.43%. Comparing the results from the three routes of administration, IM injection increases the peak of blood concentration and prolongs the $T_{1/2}$ value. Therefore, the IM administration method can be considered as a reliable route for roIFN-τ administration.

ELISA and CPE inhibition bioassay are two common methods for quantitatively measuring IFN concentration. Usually, ELISA is a rapid and simple way to determine the protein concentration. Nevertheless, regarding roIFN-τ, it cannot determine the serum bioactivity as rBoIFN-α in due course. More significantly, at the present time, there is no available roIFN-τ ELISA kit approved by a competent authority. Consequently, CPE inhibition bioassay is used in the current study to quantitatively detect the bioactivity of roIFN-τ in lamb sera according to the descriptions of several published articles (Armstrong 1981, Familletti et al. 1981, Iwata et al. 1996).

To conclude, we have compared three administrative methods for roIFN-τ injection to investigate its PKs in lambs. Our study reveals that the bioavailability in the IM injection group is higher than that in the SC injection group ($p<0.05$), and the half-life in the IM injection group is also highest among the three administration groups ($p<0.05$), which may suggest that IM injection is an optimal method for clinical application. On this basis, we hope that the present study will provide a contribution to the future application of roIFN-τ and the treatment for ovine viral diseases.

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