



Pharmacokinetic studies of the recombinant chicken interferon- α in broiler chickens

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ABSTRACT. In this study, 24 male and female broiler chickens at 30-day-old were divided into three groups with 8 animals in each group. The animals were administered with recombinant chicken interferon- α (rChIFN- α) at a dose of 1.0×10^6 IU/kg intravenously, intramuscularly or subcutaneously, respectively. Serum samples were collected at different time points post administration, and the titers of rChIFN- α in the blood were determined by cytopathic effect inhibition assay. The results showed that the pharmacokinetic characteristics of rChIFN- α by intramuscular injection and subcutaneous injection were fitted to one compartment open model, and the T_{max} was 3.21 ± 0.79 hr and 3.95 ± 0.85 hr, respectively, and the elimination half-life ($T_{1/2}$) was 6.20 ± 2.77 hr and 5.03 ± 3.70 hr, respectively. In contrast, the pharmacokinetics of rChIFN- α via intravenous injection was in line with the open model of two-compartment and was eliminated in the first order, and the elimination half-life ($T_{1/2}$) was 4.61 ± 0.84 hr. In addition, compared with those in the intravenous group and the subcutaneous group, the bioavailability of rChIFN- α in the intramuscular group was 82.80%. In conclusion, rChIFN- α was rapidly absorbed and slowly eliminated after intramuscular administration of single dose of rChIFN- α aqueous formulations. Thus, rChIFN- α can be used as a commonly-used therapeutic agent.

KEY WORDS: bioavailability, chicken interferon- α , cytopathic effect inhibition assay, pharmacokinetics

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Interferons (IFNs) are the first line of cellular innate defense against viral infection. There have been a number of studies on the inhibition of replication of viruses by IFN [17, 26]. These inducible cytokines, which have a wide variety of effects on various cell types and functions, participate in the complex cytokine network that amplifies and regulates the cellular functions involved in the immune response to pathogens [10, 21]. IFNs belong to the class II cytokine family and are divided into three classes according to the receptor complex, designated as IFN-I, IFN-II and IFN-III. Type-I IFNs in humans include IFN- α , - β , - ϵ , - κ and - ω , and they play important roles in inhibition of viral replication and cell growth [23]. Type II IFN consists of a single type, IFN- γ , which plays important roles in adaptive immune response and is crucial in the activation of natural killer (NK) cells and macrophages [7, 28]. Type III IFNs, also noted as IFN- λ , were recently identified as a member of the Interleukin-10-related cytokine family, but displays type I IFN-like antiviral activity [2]. Each class of IFNs has many different effects, though some effects are overlapped [22].

The antiviral activities of chicken IFN- α (ChIFN- α) have been documented in response to infections by Newcastle disease virus (NDV) [11], infectious bronchitis virus [15], infectious bursal disease virus [13], Marek's disease virus [9], Rous sarcoma virus [16] and influenza viruses including the Avian influenza A/H9N2 virus [20, 27]. There are numerous studies demonstrating the antiviral activity and adjuvant function of recombinant chicken IFN- α (rChIFN- α) in different infection models, suggesting that rChIFN- α could be a potential antiviral agent in the control of those virus infections.

To our knowledge, no study has been reported on the pharmacokinetics of ChIFN- α in broiler chickens. In this study, we aimed at the investigation of the pharmacokinetics of recombinant chicken IFN- α in broiler chickens. The recombinant chicken IFN- α was produced by recombinant prokaryotic expression through genetic engineering technology. The rChIFN- α was administered by intravenous, intramuscular or subcutaneous injection, respectively, and the results showed the effect of treatment and emergent prevention of viral infections, such as Newcastle disease in chickens. Our data showed that intramuscular (i.m.) injection of

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rChIFN- α yielded excellent bioavailability, suggesting that i.m. injection is the potential and feasible route for the application of the rChIFN- α . The objectives in this study were: 1) to document the pharmacokinetic behavior of rChIFN- α after a single intravenous or subcutaneous or intramuscular dose and 2) to evaluate the bioactivity of rChIFN- α in serum at various time points after drug administration by an *in vitro* bioassay. This study is original in that it provides a detailed evaluation of the parameters investigated following administration by intravenous route. We hope that the results obtained will contribute to scientific research of the pharmacokinetics of rChIFN- α .

MATERIALS AND METHODS

Animals and materials

Twenty-four broiler chickens at 30-day-old, half male and half female, were used in this study. The strain of the animal was Arbor Acres broiler chickens. All chicks were obtained from a commercial hatchery at 1 day of age. Animals were fed *ad libitum* with commercial food for 30 days, and the chick breeder was Research Animal Center of Anhui Province (Hefei, China). Animals were weighed individually with the range from 756 to 860 g (800 ± 100 g), and they were randomly divided into 3 groups with 8 chickens in each group.

The rChIFN- α lyophilized powder for injection were provided by the Anhui Jiuchuan Biological Technology Co., Ltd. (Batch number: 20110901, Wuhu, China) which was produced by recombinant prokaryotic expression as previously described [12]. The product was pretreated with ToxinEraser™ Endotoxin Removal Kit (GenScript, Piscataway, NJ, U.S.A.) according to manufacturer's instruction to remove possible LPS contamination. The resulting rChIFN- α was purified, sterilized and lyophilized. The titer of the product was 1.1×10^7 IU /vial.

Animals in group 1 were received rChIFN- α intravenously at a dose of 1.0×10^6 IU/kg. Chickens in group 2 were administered with the same dose of rChIFN- α intramuscularly, and those in group 3 were given the same dose of rChIFN- α subcutaneously. Control animals were given saline through intravenous, intramuscular or subcutaneous injection, respectively.

Sample collection

Following rChIFN- α administration, blood samples were collected from the brachial wing vein of all the animals at 0, 0.25, 0.50, 1, 2, 4, 6, 8, 12, 24, 30, 36 and 72 hr [8]. Serum was obtained by centrifugation of clotted blood at 3,000 rpm for 10 min within 2 hr after blood collection and stored at -70°C . The protocol for the animal experiment in this study was approved by the Ethics Committee of Anhui Medical University.

rChIFN- α analysis in serum

IFN titer was determined by cytopathic effect (CPE) inhibition assay in vesicular stomatitis virus (VSV) infected chicken embryo fibroblasts (CEF). In brief, primary CEF cells were seeded in 96-well microplates at 3×10^4 cells per well and cultured in MEM medium at 37°C in humidified air with 5% CO_2 for 10 hr. Monolayers of CEF cells were treated with 100 μl of four-fold serial dilutions of rChIFN- α . After 24 hr of incubation, the cells were challenged with 100 TCID₅₀ (50% tissue culture infective dose) of VSV per well and cultured until the CPE of virus-infected cells (no rChIFN- α treatment) appears. Cultures were stained with crystal violet before the plaques were counted. One interferon unit was defined as the highest dilution of ChIFN- α that inhibited 50% of the CPE at the time that 100% CPE was observed in no IFN treated wells. The rChIFN titers (IU) are expressed as the reciprocal of the dilutions that lead to 50% virus-induced cells lysis by the Reed-Muench method [19]. A recombinant human IFN- α (rhIFN- α 1, 3×10^6 IU/ml, batch number 97/04) was obtained from Institute of Food and Drug Identification of China (Beijing, China) and was used as a positive control in the titering. The precision expressed as % RSD of the interferon standard was 1.2%, and the accuracy of the interferon standard expressed as Relative Mean Error (RME) was $\leq 10.16\%$. In our study, the results of precision and accuracy fulfilled the requirements.

Data processing and statistical analysis

The results of rChIFN- α titer were analyzed by the average \pm standard deviation ($\bar{x} \pm \text{SD}$). The data from each time point of 5 blood rChIFN- α concentrations after intravenous, intramuscular and subcutaneous administrations were calculated by curve-fitting with DAS software and WinNonlin (version 3.1; Pharsight Corp., St. Louis, MO, U.S.A.) software package using noncompartmental analyses. Standard pharmacokinetic parameters, including area under plasma concentration–time curve (AUC (IU /l*hr)); clearance (CL (l/hr)); maximum plasma concentration (C_{max} ($\times 10^4$ IU/l)); elimination rate constant (K_{10} (1/hr)); elimination half-life ($t_{1/2}$ (hr)); absorption half-life ($t_{1/2ka}$ (hr)); time to peak concentration (t_{max} (hr)); and volume of distribution (V_d (l)) were calculated. Serum concentration data were uniformly weighted for these analyses, and the AUC after subcutaneous dosing was calculated using the linear-up/log-down trapezoidal method. For purposes of calculating AUC_{0- ∞} and clearance, a terminal rate was determined using the slope up to 48 hr. Data were analyzed by unpaired two-tailed *t*-test to compare antiviral activity in serum collected each day after drug administration from drug-treated animals and saline-treated control animals. Statistical significance was set at $P < 0.05$.

The equation of bioavailability was: $\text{bioavailability} = F = (\text{AUC}_{\text{s.c.}} \text{ or i.m.} \times \text{D}_{\text{i.v.}}) / (\text{AUC}_{\text{i.v.}} \times \text{D}_{\text{s.c.}} \text{ or i.m.}) \times 100\%$

Because the pharmacokinetics of rChIFN- α in plasma showed two elimination phases after intravenous injection, two-compartment model was selected, and the equations of volume of distribution were: V_d of steady-state ($V_{\text{dss}} = (k_{12} + k_{21}) / k_{21} \times V_1$). (V_1 means the distribution volume of compartment 1, $V_1 = \text{Dose} / C_p^0$)

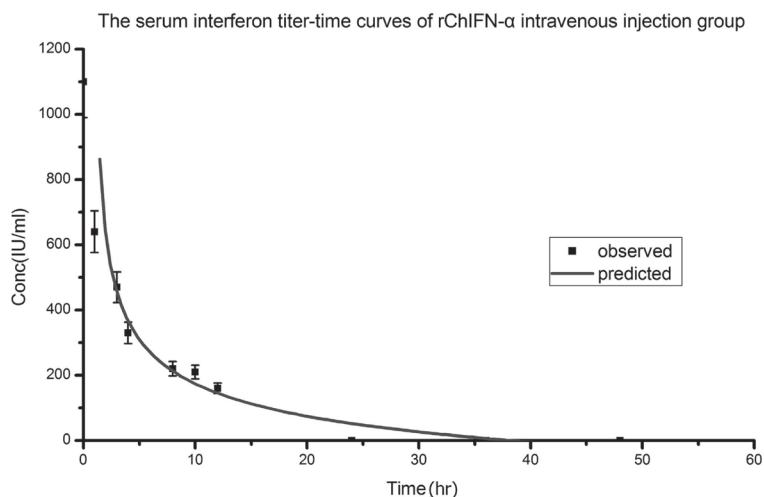


Fig. 1. The serum interferon titer-time curves of rChIFN- α intravenous injection group. The X axis was the time, and the Y axis was the titer of chicken interferon- α . The scattered square represented the mean values, and the Y-error bar represented the standard deviation. The number of observations of chickens in intravenous injection group was eight.

Table 1. The main pharmacokinetic parameters of rChIFN- α for injection (n=8, $\bar{x} \pm SD$)

Parameter	Intravenous injection group	Intramuscular injection group	Subcutaneous injection group
$T_{1/2\beta}$ (hr)	4.61 ± 0.84	6.20 ± 2.77	5.03 ± 3.70
$T_{1/2ka}$ (hr)	-	1.04 ± 0.58	1.65 ± 1.32
CL (l/hr)	187.61 ± 18.43	-	-
AUC (IU //*hr)	$4,419.60 \pm 640.49$	$3,654.41 \pm 1,003.88$	$1,186.71 \pm 279.66$
K10 (1/hr)	3.19 ± 0.58	-	-
T_{max} (hr)	-	3.21 ± 0.79	3.95 ± 0.85
C_{max} ($\times 10^4$ IU //)	$9,587.42 \pm 56.64$	363.06 ± 38.46	94.87 ± 10.23
Vdss (l)	0.40 ± 0.02		

RESULTS

Experimental chickens were received rChIFN- α at 1×10^6 IU/kg through intravenous, intramuscular or subcutaneous injection, respectively, and blood rChIFN- α potency was determined by its anti-viral effect in VSV-infected cell cultures.

The pharmacokinetic results of rChIFN- α in tested animals are shown in Table 1. The pharmacokinetic characteristics of rChIFN- α by intravenous injection were in line with the open model of the two-compartment and was eliminated in the first order, and the $t_{1/2}$ was 4.61 ± 0.84 hr (Fig. 1).

The pharmacokinetic characteristics of rChIFN- α by both muscle injection and subcutaneous injections were fit to one compartment open model, and T_{max} was 3.21 ± 0.79 hr and 3.95 ± 0.85 hr, respectively, and the elimination half-life was 6.20 ± 2.77 hr and 5.03 ± 3.70 hr, respectively (Figs. 2 and 3).

The bioavailability of subcutaneous administration of the drug in this study was 26.85%, and that of intramuscular administration of the drug was 82.80%. The distribution volume of the intravenous injection was 0.40 ± 0.02 l.

There was no statistically significant difference in gender of the animals in pharmacokinetics parameters at any dose level (data not shown). There were no safety issues during or after treatment with any doses of the rChIFN- α .

DISCUSSION

In 1957, Isaacs and Lindenmann published their first paper on interferon in which the two scientists were investigating a well-known phenomenon “viral interference”, and cells infected with one virus were able to inhibit the infection by a second virus [5]. It was shortly determined that, rather than being mediated by a component of the first virus as they initially hypothesized, the interference instead was mediated by a soluble cellular factor that could be transferred to an uninfected culture and confer virus-resistance on that culture. This discovery of the substance which they termed “interferon” provided the impetus of the development of the field of interferon biology that continues to be vigorous to the present. Interferon, like most of the described cytokines, was

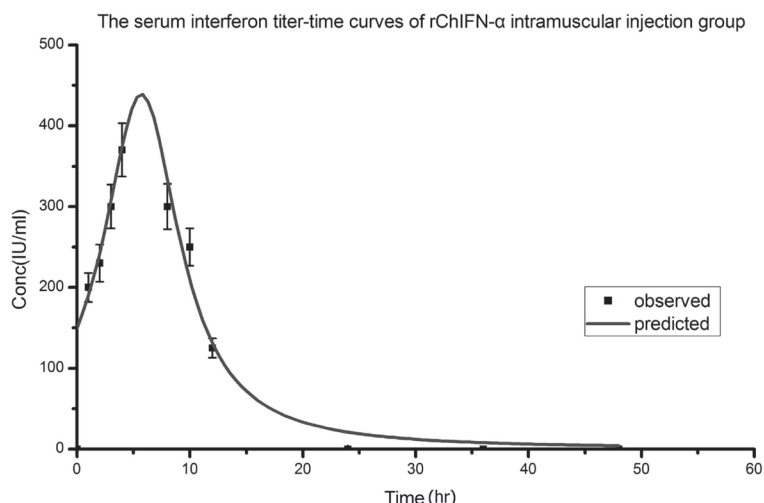


Fig. 2. The serum interferon titer-time curves of rChIFN- α intramuscular injection group. The X axis was the time, and the Y axis was the titer of chicken interferon- α . The scattered square represented the mean values, and the Y-error bar represented the standard deviation. The number of observations of chickens in intramuscular injection group was eight.

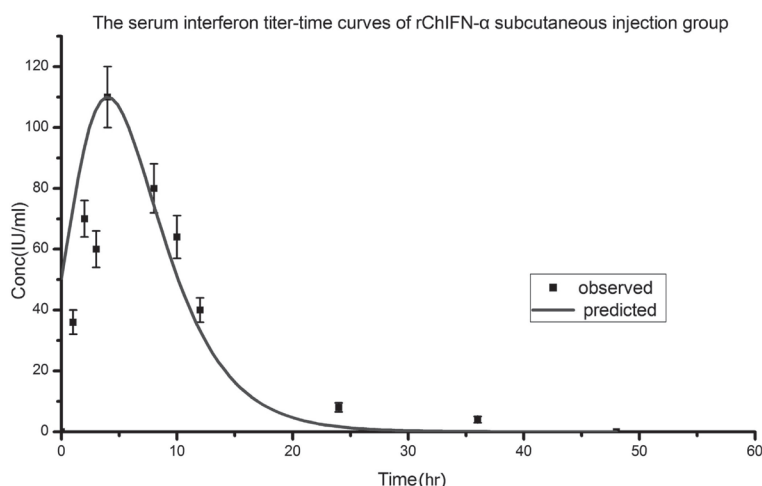


Fig. 3. The serum interferon titer-time curves of rChIFN- α subcutaneous injection group. The X axis was the time, and the Y axis was the titer of chicken interferon- α . The scattered square represented the mean values, and the Y-error bar represented the standard deviation. The number of observations of chickens in subcutaneous injection group was eight.

named for its first observed biological function-inhibition of viral infection.

IFN displays species-specificity; therefore, studies on its toxicology, pharmacokinetics and general pharmacology have encountered species-specific problems [3]. Although pharmacokinetics has to be fully illustrated before it could be used clinically, no study on the pharmacokinetics of Chicken IFN has been reported.

To date, the clinical application of interferons has been hampered by an incomplete understanding of their mechanism of action. However, there is supporting evidence that the route of administration, i.e. the pharmacokinetic behavior, is an important treatment variable. The pharmacokinetics of interferons have been fairly well described. The decline in serum concentrations of interferon is rapid shortly after its intravenous administration. The volume of distribution approximates 20 to 60% of bodyweight. Researches in animals suggested that the catabolism of interferons falls within the natural handling of proteins. Clearance values vary (4.8 to 206 l/hr) across the family of interferons which probably reflect the natural internal digestion and turnover of the proteins. Terminal elimination half-lives range from 4 to 16 hr for interferon- α . Intramuscular and subcutaneous administrations results in protracted, but fairly good absorption-greater than 80% for interferon- α [25].

Interferon therapy in human has been associated with adverse events which are usually mild and reversible. Temporal relationships exist between the degree and duration of adverse effects and the route of administration. Attempts to relate inducible

biochemical markers, such as 2',5'-oligoadenylate synthetase activity, to dose or concentration have met with some success, although alterations in these markers have not been shown to relate to clinical response [25].

The decrease of IFN- α concentrations in serum was associated with increases in CL and V_{SS} . IFN- α is quickly eliminated from the body via several pathways. The major route of IFN- α excretion is through kidney, and renal tubular cells can extract and break down many plasma proteins. IFN- α is also internalized and catabolized intracellularly in the kidney via receptor-mediated endocytosis. Various immune cells express the receptors for IFNs and may contribute to the elimination of IFNs [24]. The number of IFN receptors on mononuclear cells decreases by 42 to 80% during IFN treatment. IFNs have the inhibitory effect of P450 enzymes. Both factors may contribute not only to the decrease in IFN concentration but also to the increase [24].

The metabolism of recombinant interferon- α is consistent with that of interferon- α in general [18]. Interferons- α in blood is filtered through the glomeruli and undergoes rapid proteolytic degradation during tubular reabsorption, rendering a negligible reappearance of intact interferon- α in the systemic circulation. Only small amount of radiolabeled recombinant interferon- α appeared in the urine of isolated kidneys, suggesting near complete reabsorption of recombinant interferon- α catabolites [18]. Interferon- α was shown metabolized primarily in kidney and liver, and negligible amounts of IFN are excreted in the urine. Liver metabolism and subsequent biliary excretion are considered minor pathways for elimination of interferon *in vivo*.

The pharmacokinetic parameters following a single subcutaneous dose of rChIFN- α in chickens were determined in this study (Table 1), which was similar to those in rabbits, rats and monkeys. The administration of rChIFN- α displayed a characteristic biphasic elimination profile. The terminal elimination half-life ($t_{1/2}$) of rChIFN- α ranged from 1.33 hr to 8.73 hr in chickens. Also, its half-life was similar to that observed for human IFN- α [14].

The absorption of rChIFN- α after intramuscular injection was faster than that after subcutaneous injection. AUC in circulating blood of the subcutaneous injection group was 0.32 times of that of intramuscular injection group or about 1/3 of AUC i.m. This phenomenon may be explained by the two possible reasons. Firstly, due to the existence of various proteases in subcutaneous tissue, protein and polypeptide drugs could be partly degraded by such proteases after subcutaneous injection, so generally hypodermic bioavailability of protein or peptide drugs including rChIFN- α is not high. Secondly, for the reason that the subcutaneous administration usually involves multi-point injections, the low AUC with the sc injection may partly come from the active biochemical metabolism at those multiple injection sites. This observation suggested that at the same dose, the peak of the drug in the circulation by subcutaneous administration was lower than that by intramuscular administration, and the uptake by subcutaneous administration was also lower than that by intramuscular administration. Thus, subcutaneous administration of rChIFN- α in the blood circulation has maintained at a lower concentration levels.

The kinetics of rChIFN- α after intravenous injection was in line with the open model of the two-compartment, and the protein was eliminated in the first order kinetics, and the elimination half-life ($T_{1/2\beta}$) was 4.61 ± 0.84 hr. The rChIFN- α by intravenous administration was absorbed quickly, eliminated faster and rapidly disseminated *in vivo*, and therefore, multiple small dosages of the drug or continuous infusion need to be considered in order to maintain required blood concentration. The bioavailability of rChIFN- α by muscular injection was 82.80%, and at the same time, the muscle administration reached the peak fast, and the $T_{1/2}$ was prolonged, and therefore, it was considered a reliable method for drug delivery.

ELISA and cytopathic effect inhibition assay are the two common methods for quantitative detection of IFN. ELISA can quantitatively determine the protein concentration of rChIFN- α quickly and easily; however, it does not show its bioactivity, and more importantly, there is no validated ChIFN- α ELISA kit approved by the competent authorities. Therefore, our study used cytopathic inhibition assay to quantitatively detect the bioactivity of rChIFN- α in animal serum, which was developed based on a few published methods [1, 4, 6].

Our pilot experiments showed that the effective dose for rChIFN- α was 1×10^3 U/ml. Our practical administration schedule for rChIFN- α in chickens was 1,000 U per adult chicken per day for 3 days, with no extra dosage. If after consecutive 3 day treatment, the clinical appearance of the chickens showed no improvement, rChIFN- α will be injected again.

In conclusion, we compared three dosing routes of administration of rChIFN- α to study the pharmacokinetics in broiler chickens. We showed that the bioavailability in the intramuscular injection group was higher than that in the subcutaneous group, and the half-life in the intramuscular group was longer than that in the intravenous group, which may indicate that intramuscular administration is a better way in clinical practice. The analysis in our research may provide a basis for the clinical application of rChIFN- α and the treatment of avian influenza virus.

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