Pharmacokinetic Properties of Recombinant Feline Interferon and Its Stimulatory Effect on 2', 5'-Oligoadenylate Synthetase Activity in the Cat

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ABSTRACT. The pharmacokinetic behavior of recombinant feline interferon produced in silkworm infected with recombinant baculovirus harboring cDNA coding for feline interferon was studied in vivo in cats. The decreasing profile of the serum interferon level after intravenous administration was fitted to a two-compartment model. The half-times of the first phase (distribution phase) and second phase (metabolic phase) were 5.0 ± 0.5 min and 31 ± 5 min, respectively. In the whole body autoradiogram, at 15 min after the administration, the highest radioactivity was observed in urine in the bladder, and predominant radioactivity in the kidneys, liver, thyroid gland and spleen. Almost no radioactivity was detected in the brain or fat. Three hr after administration, the highest radioactivity was recorded in the thyroid gland, urine in the bladder, intestinal contents, and gastric mucous membrane. The data obtained in this study suggest that recombinant feline interferon has similar pharmacokinetic properties to human interferons and that it is distributed primarily in the liver and kidneys, is catabolized rapidly mainly in the kidneys, and is excreted in the urine without residual accumulation in the body. It was confirmed that 2', 5'-oligoadenylate synthetase activity was increased by the interferon in vivo for 3 days after an intravenous bolus injection in cats.—KEY WORDS: autoradiography, feline, interferon, pharmacokinetics, 2', 5'-oligoadenylate synthetase.


Interferon (IFN) is one of cytokines detected by its antiviral activity. Human interferons (HuIFNs) have been widely used in treating human hepatitis B and C, malignant melanoma, brain tumor, hairy cell leukaemia, and so on.

Stable mass production of recombinant feline interferon (rFeIFN) has been made possible recently by making use of silkworms infected with recombinant baculovirus which contains cDNA encoding FeIFN by Yanai et al. [20, 21] for the therapeutic application to feline viral and cancer diseases. rFeIFN is a glycoprotein, stable at pH 2 and has a molecular weight (MW) of 25 kilodaltons, and its amino acid sequence, consisting of 170 amino acids, has 60% homology to those of HuIFN-α and ω, and 35% homology to HuIFN-β. rFeIFN had an antiviral action in vitro against feline herpesvirus (FHV), feline calicivirus (FCV), feline leukaemia virus, feline infectious peritonitis virus and feline immunodeficiency virus; and clinical symptoms experimentally caused by FHV and FCV were markedly reduced by rFeIFN in specific pathogen-free cats [19]. These data indicate that rFeIFN is a typical type I IFN. This report adds another typical property: the inductive increase in 2', 5'-oligoadenylate (2-5A) synthetase activities in vivo which is involved in the antiviral state of IFN-treated cells [9].

In the case of HuIFNs, pharmacokinetic behavior has been intensively investigated for experimental therapeutic use in small animals by titration of IFN activity in organs and whole body autoradiography, and clinically also. Bocci [1] extensively reviewed many reports and concluded that HuIFNs, just like other protein drugs, e.g. interleukin 2, is distributed in the kidneys and liver at an early stage and most of it is excreted from the kidneys.

We investigated some pharmacokinetic functions of rFeIFN in vivo in the cat by titration of rFeIFN activities in serum and urine and by whole body autoradiography of the cat itself with labeled 125I-rFeIFN.

In this paper, we suggest that rFeIFN is quickly distributed, is metabolized, and is excreted in exactly the same way as HuIFNs.

MATERIALS AND METHODS

Interferons: The clinical formulation of a lyophilized rFeIFN sample named KT-80 contained in a vial 5 mg of gelatin, 1 mg of sorbitol and 105 units (U) of rFeIFN which was produced in the body of
silkworms infected with recombinant baculovirus rBNV100 harboring DNA coding for FeIFN and purified [14, 21] to more than 95% purity. The rFeIFN which was radioiodinated for the study of whole body autoradiography was further purified by Red Sepharose (Pharmacia LKB Biotechnology) chromatography to 97% purity in protein content.

Biological assay of rFeIFN: Antiviral activity in the serum or urine from cats was determined by a cytopathic effect inhibition method [4] using Clandell feline kidney (CRFK) cell, American Type Culture Collection number CCL 94 and vesicular stomatitis virus, because these samples had an inhibitory effect on the propagation of Felis catus 9 (Fe9) cells. The lower limit of detectable activity in the bioassay was 10 U/ml in the serum and 50 U/ml in the urine.

Animals: IC: Fec Eur (Tif) cats were used for these studies of pharmacokinetics, autoradiography and 2-5A synthetase activity, and Japanese domestic cats for the excretion study.

Administration of rFeIFN: The rFeIFN samples were administered intravenously through the cephalic vein in a bolus at a dose of 5 × 10^6 U/kg.

Serum Preparation: Blood was collected from the cephalic vein. The serum was prepared by centrifugation for 20 min at 3,000 rpm after incubation at room temperature for 20 min, and was stored at −80°C.

Pharmacokinetic study: A pharmacokinetic study was performed by using 1 male and 2 female cats weighing 2.1–3.2 kg. No inactivation of rFeIFN activity was confirmed in vitro by incubation in the cats' sera at 37°C for 7 hr and by freeze-thawing. All of the pharmacokinetic parameters of a two-compartment model were determined by conventional means [6, 11] with a computer.

\[ C(t) = A e^{-\alpha t} + B e^{-\beta t}, \]
\[ k_{21} = (A \beta + B\alpha) / (A + B), \]
\[ k_{10} = \alpha / k_{21}, \]
\[ k_{12} = \alpha + \beta - k_{21} - k_{10}, \]
\[ V_1 = D / (A + B), \]
\[ V_2 = k_{12} / k_{21} \times V_1, \]
\[ Vd\beta = V_1 \times k_{10} / \beta, \]
\[ Vd^{\alpha} = (k_{12} + k_{21}) / k_{21} \times V_1, \]
\[ AUC = A / \alpha + B / \beta, \]
\[ TBCl = D / AUC, \]
\[ t_{1/2(\alpha)} = \ln 2 / \alpha, \]
\[ t_{1/2(\beta)} = \ln 2 / \beta, \]

where C(t) is the serum rFeIFN level at time t, and α and β are the rapid and slow disposition rate constants, respectively. A and B are the zero-time intercepts associated with the respective phases. k_{21} is the distribution rate constant out of the peripheral compartment, k_{10} is the elimination rate constant from the central compartment, k_{12} is the distribution rate constant out of the central compartment, V_1 is the apparent volume of distribution in the central compartment, V_2 is the apparent volume of distribution in the peripheral compartment, Vd\beta is the apparent volume of distribution in the slow distribution phase, Vd^{\alpha} is the apparent volume of distribution in the steady-state, TBCl is the total body clearance, AUC is the area under the serum concentration-time curve from 0 to infinity derived from model parameters, t_{1/2(\alpha)} is the half-time for rFeIFN in the first phase, and t_{1/2(\beta)} is the half-time for rFeIFN in the second phase.

125I-rFeIFN: Radioiodination of rFeIFN was performed with Na125I (New England Nuclear) by lactoperoxidase with Enzymonbeads (Bio-Rad Laboratories) according to the procedure described in the appended manual. 125I-rFeIFN was purified through gel chromatography with Sephadex G25 (Pharmacia LKB Biotechnology) precoated with rFeIFN. The radiochemical purity of the 125I-rFeIFN prepared was found to be 95.1% by the trichloroacetic acid (TCA) precipitation method [2] and its specific activity was 438 MBq/mg protein. Just before administration, cold rFeIFN, gelatin and sorbitol, 0.5% and 0.1%, respectively, were mixed with 125I-rFeIFN to 5 × 10^6 U/ml.

Autoradiography: Whole body autoradiographic studies were carried out on female cats weighing 1.6 and 1.8 kg. At the indicated times after administration, the animals were killed with CO2 in the desicicator with dry ice under anesthetization with Ketamine hydrochloride, and were rapidly frozen by immersing in acetone-dry ice. Thirty five μm sagittal whole body sections were obtained with a cryomicrotome (PMV 450MP, LKB) at −25°C from the bodies which were fixed in 5% carboxymethyl cellulose and subjected to exposure to Konica New A film at 4°C for 28 days.

Excretion studies: Urinary and fecal excretion of rFeIFN was examined by bioassay in 3 male cats weighing 2.8–3.4 kg after administration. Whole urine in the bladder was collected by catheterization of the bladder before and after dosing. The urine was mixed with the same volume of the culture
medium for CRFK cells supplemented with 10% fetal calf serum, frozen with dry ice immediately after collection, and stored at -80°C. After thawing, the pH of the urine samples was adjusted to pH 7.7 with CRFK cells for the bioassay. Collected feces were suspended in the same weight of culture medium as for CRFK cells, then frozen with dry ice and kept at -20°C. After centrifugation the supernatant was assayed for rFeIFN activity.

Quantitation of 2', 5'-oligoadenylate synthetase activity: The 2-5A synthetase activity was quantitated in vivo both in the white blood cells and in the serum from one male and two female cats. White blood cells were collected with Mono-Poly Resolving Medium (Flow Laboratories) according to the procedure described in the attached manual [5] from blood collected at the times indicated. The cell free extract of white blood cells was prepared by freeze-thawing and sonic oscillation, followed by centrifugation. The activity of 2-5A synthetase was quantitated by Special Reference Laboratory (Tokyo) on a commercial basis with a 2-5A Eiken Radioimmunounassay kit (Eiken Kagaku Ltd.). The 2-5A synthetase activity is shown as f mol/μg protein or pmol/dl serum.

RESULTS

Pharmacokinetics: As shown in Fig. 1, within 2 hr the rFeIFN level in the serum decreased biphasefully and the decrease fitted a two-compartment model. Pharmacokinetic parameters calculated in a computer are shown in Table 1. The half-times of the first phase and second phase were 5.0 ± 0.5 min and 31 ± 5 min. The Vd∞ was 111 ± 24 ml/kg, and barely larger than the estimated volume of blood.

Distribution study: The radiochemical purity of 125I-rFeIFN decreased to 91% and to 83% after 3 days, and 8 days, incubation at 4°C, respectively.

Based on this result, 125I-rFeIFN was administered 18 hr after the 125I-rFeIFN preparation. No inactivation of rFeIFN, as indicated previously in the case of HuIFN-α [2, 10], occurred in the 125I-radioiodination procedure. Autoradiography was carried out at 15 min and 3 hr after dosing with the 125I-rFeIFN sample because Fig. 1 suggested that 90% of the rFeIFN disappeared from serum was and distributed to organs at 15 min after dosing and that, at 3 hr, the rFeIFN concentration decreased less than one thousandth.

As shown in Fig. 2, at 15 min the highest radioactivity was recorded in urine in the bladder, and predominant radioactivity was detected in the kidneys, liver, thyroid gland and spleen. Heart, lungs, bone marrow and the adrenal gland contained a much lower level of radioactivity. Almost no radioactivity was detected in the autoriadogram in the brain, or fat. At 3 hr, the thyroid gland, urine in the bladder, intestinal contents, and gastric mucous

![Graph](image.png)

**Fig. 1.** Serum level of rFeIFN activity after intravenous administration; ●: Cat # 1; ○: cat # 2; +: cat # 3. Points are experimental data, and lines are simulation curves fitted to a two-compartment model.

<table>
<thead>
<tr>
<th>Cat#</th>
<th>α</th>
<th>β</th>
<th>k12 (h⁻¹)</th>
<th>k21</th>
<th>k10</th>
<th>V1</th>
<th>V2</th>
<th>Vd∞ (m/kg)</th>
<th>AUC (U/ml)</th>
<th>TBCL (m/l/min/kg)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>9.03</td>
<td>1.72</td>
<td>0.76</td>
<td>1.93</td>
<td>8.06</td>
<td>4.61</td>
<td>0.40</td>
<td>104</td>
<td>41</td>
<td>145</td>
</tr>
<tr>
<td>2</td>
<td>7.18</td>
<td>1.08</td>
<td>0.54</td>
<td>1.19</td>
<td>6.53</td>
<td>5.79</td>
<td>0.64</td>
<td>75</td>
<td>34</td>
<td>109</td>
</tr>
<tr>
<td>3</td>
<td>9.35</td>
<td>1.41</td>
<td>0.77</td>
<td>1.57</td>
<td>8.42</td>
<td>4.45</td>
<td>0.49</td>
<td>52</td>
<td>25</td>
<td>77</td>
</tr>
<tr>
<td>Mean</td>
<td>8.52</td>
<td>1.41</td>
<td>0.69</td>
<td>1.56</td>
<td>7.67</td>
<td>4.95</td>
<td>0.51</td>
<td>77</td>
<td>34</td>
<td>111</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>0.83</td>
<td>0.23</td>
<td>0.09</td>
<td>0.26</td>
<td>0.71</td>
<td>0.52</td>
<td>0.08</td>
<td>19</td>
<td>6</td>
<td>24</td>
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</tbody>
</table>

Table 1. Pharmacokinetic parameters of rFeIFN following intravenous administration.
Fig. 2. Whole body autoradiograms showing distribution of radioactivity 15 min and 3 hr after intravenous administration of $^{125}$I-IFN.
membrane had the highest radioactivity, and the esophagus mucous membrane and pharyngeal mucous membrane had high radioactivity.

**Excretion study:** To exclude experimental artifacts, no inactivation of rFeIFN was confirmed in a stability test by incubating in the urine of a cat at 37°C for 7 hr and by freeze-thawing. No rFeIFN activity more than the detectable lower limit of 50 U/ml was observed in urine 30 min, 1, 2, 4, 8 or 24 hr after rFeIFN administration.

No rFeIFN activity was detected in feces from 1 to 7 days after rFeIFN administration. However, rFeIFN activity disappeared rapidly at 37°C after mixing with supernatant filtrated through 0.2 μm sterile filter (Gelman Science) from a feces suspension at 37°C, which suggested that it may be impossible to quantitate rFeIFN activity excreted into feces.

**2’, 5’-oligoadenylate synthetase activity:** The induction of 2-5A synthetase by rFeIFN in vivo was checked, because the intracellular enzyme is supposed to play a key role in the antiviral state of IFN-treated cells [9]. The time courses of enzyme activity which increased in the white blood cells and in serum after intravenous (iv) administration of rFeIFN are shown in Fig. 3. The activity in white blood cells was increased 7 to 11 times by rFeIFN administration, and the serum level was also increased to 3 to 6 times. These high levels continued for more than 3 days.

**DISCUSSION**

Both HuIFN-α and HuIFN-β disappear from the circulation in a bi- or tri-phasic manner [1]. The half-times of the first phase are most frequently not available in patients [1]. Those of HuIFN-α and HuIFN-β are 3.3 min [17] and 15 min [16] in rats. The half-times of the second phase of HuIFN-α and HuIFN-β are less than 2 hr and 13 min, respectively, in patients [1]. The rFeIFN disappeared from the serum in a bi-exponential fashion and rFeIFN distribution was analyzed with a two-compartment model after iv administration. The half-times of the first and second phases were 5.0 and 31 min, respectively. As with natural HuIFN-β administered to rabbits [15], both distribution rate constant k21 and k12 values were smaller than elimination rate constant k10, and the Vdα α value was one fourth that of Vdβ. This means that rFeIFN is hard to distribute to organs as shown in the case of HuIFN-β [16].

HuIFNs distribute preferentially to the kidneys and liver soon after administration [10, 16, 18]. The kidneys play a preponderant role in the filtration, catabolism and excretion of IFN [3], and the liver simply accomplishes its job to remove IFN-β from the circulation [1]. No HuIFN is traceable in the urine of patients [1], nor in urine in the bladder of rats [16]. A whole body autoradiogram at 15 min after 125I-rFeIFN administration showed the higher radioactivity in the kidneys and liver. Five min after the injection of 125I-HuIFN-α, the high levels of radioactivity in the kidneys and liver were due to bioactive IFN [10]. No rFeIFN activity was detected in urine in the bladder in spite of high radioactivity. These data suggest that rFeIFN is distributed primarily in the kidneys and liver soon after iv administration, is catabolized rapidly, mainly in the kidneys, and is excreted in the urine.

Iodine is known to be distributed to the thyroid gland and gastric mucous membrane [8]. Low MW degradation products of 125I-HuIFN-α are excreted from the kidneys into the blood and are at least partially secreted in the urine and also taken up by other organs [12]. So the higher radioactivities observed in the thyroid gland, urine in the bladder, intestinal contents and gastric mucous membrane at
3 hr after the administration of $^{125}$I-rFeIFN are considered to be the result of low MW metabolites of $^{125}$I-rFeIFN or freed $^{125}$I. At 3 hr after the administration of $^{125}$I-rFeIFN, the radioactivity in the kidneys and liver was low. These data could explain one of the pharmacokinetic properties of rFeIFN, i.e. rFeIFN is rather easy to eliminate.

HulIFN concentrations in muscle and brain are either not detected or are extremely low after an iv bolus injection [7, 8]. In autoradiograms after iv administration of $^{125}$I-rFeIFN, no radioactivity was found in the brain or cerebro-spinal fluid, which suggests that rFeIFN does not pass the blood-brain barrier, and trace radioactivity was observed in muscle.

Uemura et al. [18] showed that the radioactivity in a fetus after intramuscular administration of $^{125}$I-HulIFN-α was extremely low compared with radioactivity in maternal plasma, and that about 70% of radioactivity in the fetus was soluble in TCA.

The induction of 2-5A synthetase activity is regarded as one of the markers of the effect of IFN [13]. The increase in the enzyme activity in vivo in this study has confirmed that the rFeIFN shares typical characteristic with type I HulIFNs along with wide spectra of antiviral activity, the MW, the conserved NH$_2$-terminal sequence, and stability in relation to pH 2. Therefore, rFeIFN can be classified as one of the type I IFNs.

The data shown above suggest that other pharmacokinetic properties of rFeIFN not tested in this study may be similar to those of type I HulIFNs.

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REFERENCES