DOI 10.1007/s10517-022-05521-3
Bulletin of Experimental Biology and Medicine, Vol. 173, No. 2, June, 2022 PHARMACOLOGY AND TOXICOLOGY

Pharmacokinetic Parameters of Oral Pegylated IFN-λ1

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 173, No. 2, pp. 188-192, February, 2022
Original article submitted February 7, 2022

We studied the pharmacokinetics of a pegylated IFN-λ1 (PEG IFN-λ1) after its oral administration to rats in different therapeutic doses. The hypothesis on linear pharmacokinetics of PEG IFN-λ1 within the dose range of 2.6-7.8 μg/kg was confirmed, high for protein molecules bioavailability from 17.5 to 21%, the absence of intravascular deposition, and effective elimination with feces and urine (85 and 15% of the administered dose, respectively) were demonstrated. At the same time, the mean retention time for PEG IFN-λ1 in the circulation is 6.46-6.65 h and half-life is 3 h. These findings give ground for continuing experimental studies of PEG IFN-λ1 pharmacokinetics, in particular, tissue distribution of the drug.

Key Words: *interferon lambda; pegylated interferon lambda 1 (PEG IFN-λ1); pharmacokinetic*

IFNs lambda (IFN-λs) discovered in 2003 by two independent groups of researchers [12,14] form a family of type III IFNs and are of great interest because they exhibit antiviral activity and maintain the balance between the protective and pathological immune responses [1]. The virus-protective effect of IFN-λ, particularly IFN-λ1 (IFN-λ1 or IL-29), is especially evident at the epithelial barriers [13]. IFN-λ1 exhibits significant and highly effective antiviral activity during both prevention of viral invasion and its eradication [3-6].

We studied pharmacokinetics of pegylated IFN-λ1 (PEG IFN-λ1) after its intragastric administration to substantiate its possible antiviral use for humans and animals.

MATERIALS AND METHODS

We used recombinant human IFN-λ1 produced by molecular genetic and biotechnological methods from the biomass of *E. coli* producer cells and immobilized on polyethylene glycol with a molecular weight of 1.5 kDa activated with an electron beam (PEG IFN-λ1) produced at the Siberian Center of Pharmacology and Biotechnology.

The experiments were carried out on sexually mature conventional outbred male rats (n=282) of the SD stock (age 6-8 weeks, body weight 300-350 g) from the Department of Experimental Biomodels of the E. D. Goldberg Research Institute of Pharmacology and Regenerative Medicine. The experiments were carried out in specialized experimental rooms of the Laboratory of Immunopharmacology of the E. D. Goldberg Research Institute of Pharmacology and Regenerative Medicine in accordance with Principles of Laboratory Practice (Order No. 199n of the Ministry of Health of the Russian Federation; April 1, 2016) and Directive 2010/63/EU of the European Parliament and of the Council (September 22, 2010; On the Protection of Animals Used for Scientific Purposes).

Pharmacokinetics of the medicinal substance was studied according to the Manual for Preclinical Studies of New Pharmacological Substances [11]. PEG IFN-λ1 dissolved in 0.2 ml PBS (MP Biomedicals) was administered intragastrically in three therapeutic doses: 2.6, 5.2, and 7.8 μg/kg. The lowest therapeutic dose (2.6 μg/kg) corresponds to the estimated therapeutic dose of the drug for humans. To test the linearity hypothesis, PEG IFN-λ1 was administered intragastrically

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0007-4888/22/1732-0215

once a day in the above doses. For evaluation of accumulation and excretion of PEG IFN-λ1, the drug was administered intragastrically once a day for 5 days in a dose of 2.6 μg/kg. For evaluation of the absolute bioavailability of PEG IFN-λ1, the drug was administered once intravenously in a dose of 2.6 μg/kg.

The blood was taken from the heart cavity after euthanasia of the animal. To separate the serum from the clot of cellular elements, the collected blood was incubated at 37°C for 30 min in a glass tube, left in a refrigerator for 1 h, and then centrifuged at 3000 rpm for 10 min. The isolated serum was transferred to plastic tubes and frozen at -(18-20)°C. In the analysis of absolute bioavailability of the drug, blood serum samples of the 6 intact animals obtained in a similar way were used as a zero point (before administration). The blood samples were taken before and in 0.25, 0.5, 1, 2, 4, 8, 16, and 24 h after PEG IFN-λ1 administration. The blood samples after a single intravenous injection were taken before and in 0.03, 0.17, 0.33, 0.5, 0.75, 1, and 2 h after PEG IFN-λ1 administration. Six animals were used for each time point. The urine and feces were collected from the studied animals over 3 days to study the accumulation and elimination of PEG IFN-λ1.

The serum concentration of PEG IFN-λ1 measured by ELISA using Human IL-29 Platinum ELISA kits (#BMS2049; Bender MedSystems GmbH) designed to measure the concentration of IFN-λ1 in a concentration range of 2-1000 pg/ml. The results were read at 450 nm on an AIFR-01 UNIPLAN analyzer of enzyme immunoassay reactions (Pikon).

To calculate the pharmacokinetic parameters, an out-of-model method of statistical moments was used [10]. The areas under the pharmacokinetic curve (AUC and AUMC) were calculated by the trapezoid method [2,8]. The results were processed by methods of variational statistics using Statistica 12.0 software (StatSoft, Inc.): the mean value for the sample and the standard error of the mean were calculated [9]. The differences were significant at $p<0.05$.

RESULTS

The dynamics of PEG IFN-λ1 concentration in the blood serum after single intragastric administration did not depend on the drug dose and was described by a bell-shaped curve with a maximum in 4 h after administration with and complete elimination by 24 h (Fig. 1). After 5-fold administration of PEG IFN-λ1 in a dose of 2.6 μg/kg (Fig. 2), the type of the pharmacokinetic curve did not change (Fig. 2), which indicates the absence of intravascular deposition of the drug and effective elimination.

Over 3 days, about 85% of the administered dose of PEG IFN-λ1 was excreted with feces (Table 1),

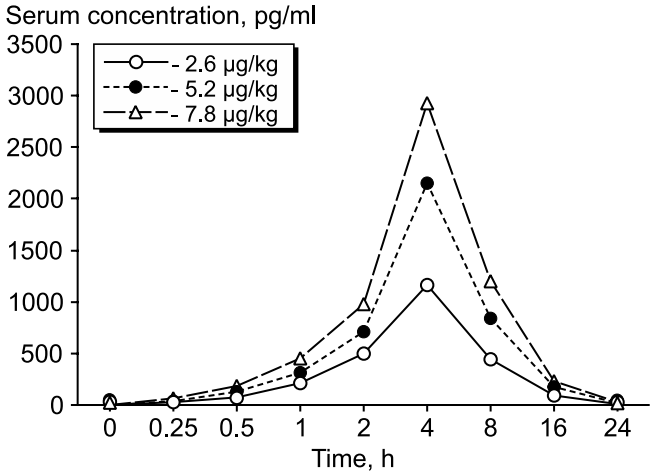

Fig. 1. Averaged pharmacokinetic profile of PEG IFN-λ1 in the blood serum of rats after single intragastric administration in different doses.

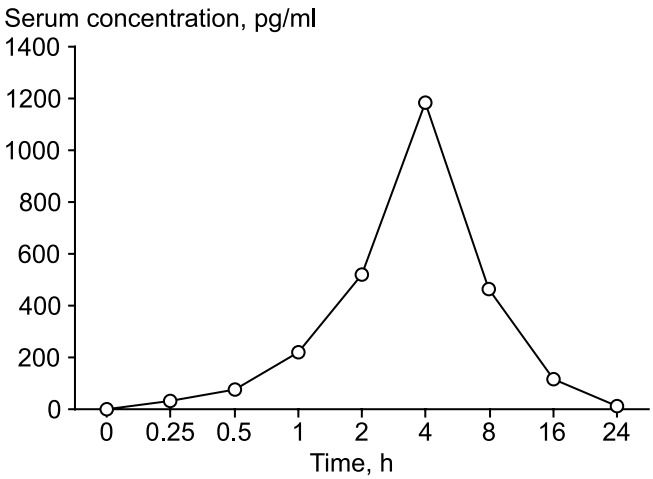

Fig. 2. Averaged pharmacokinetic profile of PEG IFN-λ1 in rat blood serum after repeated intragastric administration in a dose of 2.6 μg/kg.

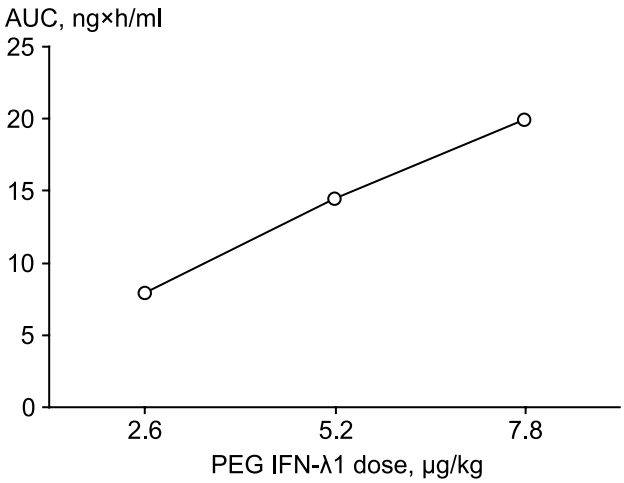

Fig. 3. AUC of PEG IFN-λ1 after single intragastric administration to rats in the studied doses.

primarily during the first 2 days; the rest 15% was excreted with the urine, primarily during the first day. On day 3, only trace amounts of PEG IFN-λ1 were detected in the urine and feces.

Analysis of the dependence of AUC values for the three doses of PEG IFN-λ1 showed that the pharmacokinetics of PEG IFN-λ1 are linear within the dose range of 2.6-7.8 μg/kg (Fig. 3). The calculated parameters of the pharmacokinetic curves of PEG IFN-λ1 in the serum after its single intragastric administration in the studied doses are shown in Table 2.

The absolute bioavailability of PEG IFN-λ1 was calculated by the formula:

$$F=AUC_{e/v}\times D_{i/v}/AUC_{i/v}\times D_{e/v},$$

where $AUC_{e/v}$ and $AUC_{i/v}$ are the areas under the pharmacokinetic curve after extravascular and intravenous administration, respectively, $D_{i/v}$ and $D_{e/v}$ are the doses for intravenous and extravascular administration, respectively. After intravenous administration of PEG IFN-λ1 in a dose of 2.6 μg/kg to rats, $AUC_{i/v}$ was 37.96 ng×h/ml, while absolute bioavailability after intragastric administration of the drug were 20.97, 19.09, and 17.54% for 2.6, 5.2, and 7.8 μg/kg PEG IFN-λ1, respectively.

Thus, in case of intragastric administration to rats, the maximum concentration of PEG IFN-λ1 increased by 2.5 times with increasing the dose of PEG IFN-λ1 by 3 times, while the time of attaining C_{max} (T_{max}) and half-elimination time remained virtually unchanged (4 and 3.02 h, respectively). The retention time (MRT) and absorption time slightly increased; the clearance and kinetic volume of distribution increased by 1.2 times, and the absolute bioavailability decreased by 16% (from 20.97 to 17.54%).

The maximum concentration of PEG IFN-λ1 after 5-fold intragastric administration in a dose of 2.6 μg/kg was 1.19 ng/ml (Table 3), which is comparable with the maximum concentration after single administration; the time of reaching the maximum concentration (T_{max}) was also 4 h (similar to single dose) and the clearance was 2.89 liter/h in comparison with 98.05 ml/h after single dose. The AUC was increased by 4.3% in comparison with single administration of PEG IFN-λ1 in a dose of 2.6 μg/kg.

Thus, the pharmacokinetic parameters of the studied drug PEG IFN-λ1 allow us to consider it as a prototype of an oral antiviral drug by a number of signs. It is well known that high-molecular compounds in their native form are poorly absorbed by the endocytotic vesicular mechanism [7]. However, PEG IFN-λ1 demonstrates the sufficiently high bioavailability for the protein molecules: from 17.5 to 21% in all studied doses (2.6, 5.2, and 7.8 μg/kg). PEG IFN-λ1 demonstrates material accumulation in the blood serum in direct dependence on the administered dose with participation of the same type of translocation mechanism. The maximum concentration was always observed in 4 h after administration with a gradient of 1.17>2.15>2.93 ng/ml. PEG IFN-λ1 persists in

TABLE 1. Content of PEG IFN-λ1 in the Urine and Feces of Rats over 3 Days after Single Intragastric Administration in a Dose of 2.6 μg/kg (*X*±*m*)

Day of the study	PEG IFN-λ1 concentration, ng/day	
	urine	feces
1	77.50±6.65	253.67±38.70
2	18.75±3.15	325.55±56.45
3	6.54±1.18	22.77±4.18

TABLE 2. Pharmacokinetic Parameters of PEG IFN-λ1 in Rat Blood Serum after Single Intragastric Administration in the Studied Doses

Parameter	Dose of PEG IFN-λ1		
	2.6 μg/kg	5.2 μg/kg	7.8 μg/kg
AUC, ng×h/ml	7.96	14.49	19.97
AUMC, ng×h²/ml	51.40	96.43	132.24
Cl, ml/h	98.05	107.64	117.16
C_{max}, ng/ml	1.17	2.15	2.93
k_{el}, h^{-1}	0.23	0.23	0.23
$T_{1/2}$, h	3.02	3.02	3.01
MRT_{po}, h	6.46	6.65	6.62
T_{max}, h	4.0	4.0	4.0
MAT, h	5.08	5.27	5.24
$V_{z\ po}$, ml	427.58	468.59	509.52

Note. Here and in Table 3: AUC is the total area under the experimental "concentration—time" curve, AUMC is the area under the curve "product of time by concentration of a pharmacological agent", Cl is clearance, C_{max} is the maximum concentration, k_{el} is the elimination constant, $T_{1/2}$ is the half—life, MRT_{po} is the mean retention time of the drug in the body after oral administration, T_{max} is the time to maximum concentration, MAT is the average absorption time, $V_{z\ po}$ is the volume of distribution of the drug after oral administration.

TABLE 3. Pharmacokinetic Parameters of PEG IFN-λ1 in Rat Blood Serum after 5-Folg Intragastric Administration in a Dose of 2.6 μg/kg

Parameter	Value
AUC, ng×h/ml	8.30
AUMC, ng×h²/ml	54.38
Cl, liter/h	2.89
C_{max}, ng/ml	1.19
T_{max}, h	4.0

the circulation for a long time: MRT is in the range of 6.46-6.65 h, $T_{1/2}$ was 3 h. PEG IFN-λ1 mainly eliminates during the first two days; on day 3, only trace amount of the drug was detected in both urine and feces; about 85% of the administered dose of PEG IFN-λ1 is excreted in feces and about 15% with the urine. These findings give ground for continuing experimental studies of the pharmacokinetics of PEG IFN-λ1 in terms of studying the tissue distribution of the drug.

The work was carried out within the framework of the State Contract No. 14.N08.12.0066.

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