Cancer Chemother Pharmacol (1993) 33: 258–261

Cancer Chemotherapy and Pharmacology

Pharmacokinetics of an extended-release human interferon alpha-2b formulation

Andrea Bonetti*, Sinil Kim

UCSD Cancer Center, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0812, USA
Dr. Bonetti is recipient of a grant from Unita' Sanitaria Locale 25, Verona, Italy

Received: 22 December 1992/Accepted: 4 August 1993

Abstract. The in vivo half-life of human interferon alpha-2b (hIFN-α-2b) is relatively short, and frequent injections over prolonged periods are required for efficacy. An extended-release formulation of hIFN-α-2b (Depo/IFN) was created by encapsulation into a lipid-based drug-delivery system. The capture efficiency was 51% ± 13% and the release half-life in human plasma at 37 °C was 16 days. The pharmacokinetics of Depo/IFN was compared with that of unencapsulated standard hIFN-α-2b (Std/IFN) in the peritoneal cavity of male BDF_1 mice. Depo/IFN exhibited a 13-fold longer intraperitoneal (i.p.) half-life as compared with Std/IFN (20 vs 1.5 h). The release of free hIFN-α-2b from Depo/IFN into the peritoneal cavity was slow and protracted, with a 10-fold lower peak concentration and a 13-fold longer apparent half-life being observed in comparison with Std/IFN. The areas under the curve of free hIFN-α-2b in the peritoneal cavity were comparable for Depo/IFN and Std/IFN. hIFN-α-2b was detectable in plasma only after the i.p. administration of Std/IFN. These data suggest the possibility that Depo/IFN may be useful as an extended-release formulation of hIFN-α-2b.

Introduction

Recombinant human interferon alpha (hIFN-α) is useful in the treatment of a broad spectrum of diseases, such as hairy-cell leukemia, chronic myelogenous leukemia, Kaposi's sarcoma, laryngeal and genital papillomas, and chronic viral hepatitis of types B and C [5]. Furthermore, the activity of hIFN-α in combination with cytotoxic agents is being actively investigated [22].

Pharmacokinetics studies in animals [3, 4, 10, 12, 18] and humans [11, 19, 21, 23] have shown that the in vivo half-life of hIFN-α is relatively short and requires frequent injections over prolonged periods. Although the relationships among hIFN-α pharmacokinetics, clinical efficacy, and toxicity are complex and incompletely characterized [24], an extended-release formulation of the drug could reduce the frequency of injections and may be of benefit in locoregional treatment.

For tumors that metastasize within the peritoneal cavity, intraperitoneal administration is potentially useful because cancer cells can be exposed to high drug concentrations with reduced systemic exposure [7]. Intraperitoneal use of hIFN-α may be useful because of such a pharmacologic advantage [20] or, perhaps, for the purpose of activating regional effector mechanisms in the peritoneal cavity [15].

Depo/IFN is an extended-release formulation of hIFN-α-2b that consists of microscopic particles that enclose multiple nonconcentric aqueous chambers, into which hIFN-α-2b is encapsulated. These particles are structurally distinct from traditional liposomes, which enclose either multiple concentric internal chambers or a single internal chamber per particle. The bilayer lipid membranes are made from nontoxic synthetic lipids identical to those found in cell membranes. This report describes the synthesis and intraperitoneal pharmacokinetics of Depo/IFN in a murine model.

Materials and methods

Materials. hIFN-α-2b was obtained from Schering Corporation (Kenilworth, N.J.). Dioleoyl lecithin, dipalmitoyl phosphatidylglycerol, and cholesterol were acquired from Avanti Polar Lipids, Inc. (Birmingham, Ala.); triolein and free-base L-lysine were procured from Sigma (St. Louis, Mo.); and nanograde chloroform was obtained from Malinckrodt (Paris, Ky.). All these reagents were used without further purification. The vortex mixer was obtained from American Scientific Products (catalogue number S8223-1, McGaw Park, Ill.). BDF_1 mice were supplied by Simonsen Laboratories (Gilroy, Calif.). Microtiter plates (96-well) were obtained from Dynatech Laboratories, Inc. (Chantilly, Va.). Monoclonal

* Present address: Department of Medical Oncology, Hospital of Borgo Trento, P. le Stefani, 1-37121 Verona, Italy

Correspondence to: Andrea Bonetti, M.D., Department of Medical Oncology, Hospital of Borgo Trento, P. le Stefani, 1–37121 Verona, Italy

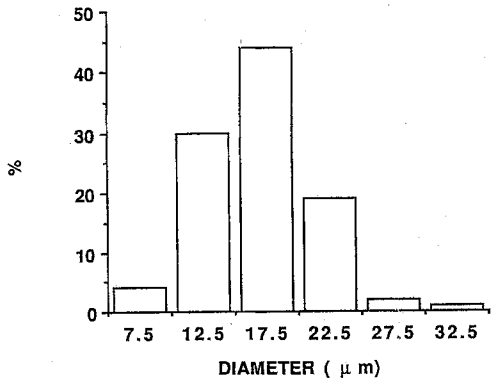

Fig. 1. Distribution of Depo/IFN as a function of particle diameter

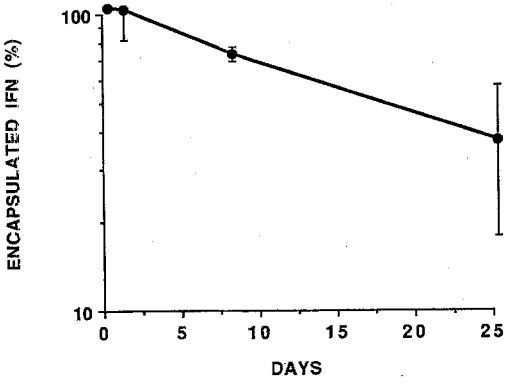

Fig. 2. Release kinetics of hIFN-α-2b from Depo/IFN in human plasma at 37 °C

anti-hIFN-α antibodies were supplied by Calbiochem (San Diego, Calif.). Rabbit anti-hIFN-α antibodies were purchased from Biosource International (Camarillo, Calif.). Peroxidase-labeled goat anti-rabbit IgG was supplied by Tago Inc., (Burlingame, Calif.). *O*-Phenylenediamine and hydrogen peroxide were purchased from Sigma (St. Louis, Mo.).

Synthesis of Depo/IFN. Depo/IFN was prepared with modifications of the previously published method for encapsulating cytarabine [14]. For each batch of Depo/IFN, 1 ml of discontinuous aqueous phase (hIFN-α-2b, 1.1×10^6 IU/ml; mannitol, 40 mg/ml; sodium acetate, 2.5 m*M*; glycine, 12 m*M*; Na_2HPO_4, 7 m*M*; NaH_2PO_4, 2 m*M*; Tween-80, 0.01 mg/ml; HCl, 0.1 *N*; human serum albumin, 0.04 mg/ml) was added into a 1-dram vial containing 13.9 μmol of dioleoyl lecithin, 3.15 μmol of dipalmitoyl phosphatidylglycerol, 22.5 μmol of cholesterol, 2.7 μmol of triolein, and 1 ml of chloroform. The final pH of the aqueous phase was 1.1. The vial was attached horizontally to the head of the vortex mixer and shaken at maximal speed for 6 min. Half of the resulting "water-in-oil" emulsion was expelled rapidly through a narrow-tip Pasteur pipette into each of two vials (1-dram), each containing 2.5 ml of water, glucose (32 mg/ml), and free-base lysine (40 m*M*). Each vial was then shaken on the vortex mixer for 5 s at maximal speed to form chloroform spherules. The chloroform-spherule suspensions in the two vials were transferred into a 250-ml Erlenmeyer flask containing 5 ml of water, glucose (32 mg/ml), and free-base lysine (40 m*M*). A stream of nitrogen gas flowing at 7 l/min was used to evaporate the chloroform over a period of 10 – 15 min at 37 °C. The Depo/IFN particles were then isolated by centrifugation at $600 \times g$ for 5 min and washed thrice with 0.9% NaCl solution. The capture efficiency or yield was determined by enzyme-linked immunosorbent assay (ELISA, as described below) of the interferon encapsulated within Depo/IFN particles and then expressed as a percentage of the initial amount of interferon used at the beginning of the procedure.

Pharmacokinetics studies. Intraperitoneal (i.p.) pharmacokinetics studies were performed using male BDF_1 mice weighing 20 – 25 g. They were injected i.p. with 30,000 IU of unencapsulated Std/IFN or Depo/IFN in 1 ml of phosphate-buffered saline (PBS) – bovine serum albumin (BSA) buffer (BSA, 10 mg/ml). Animals were anesthetized and at the 0-, 0.25-, 0.5-, 2-, 4-, and 8-h time points for the Std/IFN group and the 0-, 8-, 24-, 48-, 72-, and 96-h time points for the Depo/IFN group, 5 μl of undiluted peritoneal fluid was collected into capillary pipettes and then placed into a tube containing 120 μl of PBS-BSA buffer. The samples were centrifuged in an Eppendorf microfuge for 1 min to separate the supernatant (containing the free drug) from the pellet (containing the Depo/IFN particles). Depo/IFN particles were lysed with methanol (20% final concentration). The peritoneal cavity was then washed out thoroughly with 4 – 5 ml of PBS-BSA buffer and the washings were collected. At the same time points, blood samples were collected into heparinized tubes and the plasma was separated. All the samples were kept frozen at –20 °C until analysis by ELISA. Three animals were used at each time point.

The RSTRIP program (MicroMath Scientific Software, Salt Lake City, Utah) was used to perform pharmacokinetic calculations. A single-compartment model was used. The area under the curve (AUC) was determined by linear trapezoidal rule.

In vitro release studies. Depo/IFN release studies in human plasma at 37 °C were done according to a previously published method [14]. Briefly, a minimum of 40 vols. of blood-bank plasma was added to the washed Depo/IFN pellets. The suspension was placed in a syringe, 0.01% sodium azide was added to inhibit the growth of microorganisms, air was excluded, and the syringe was placed in a 37 °C incubator. At appropriate time points, aliquots of the suspension were removed from the syringe and centrifuged in an Eppendorf microfuge for 5 min. The supernatant was removed and methanol [20% (v/v) final concentration] was added to the pellet, which was stored at –20 °C prior to assay. The hIFN-α-2b content in the pellet was determined by ELISA and expressed as a percentage of the initial amount.

Enzyme-linked immunosorbent assay. Prior to the assay of hIFN-α-2b concentrations by ELISA, methanol [20% (v/v) final concentration] was added to the samples, which were frozen at –20 °C to disrupt Depo/IFN membranes and effect the release of interferon. Microtiter plates (96-well) were coated by the addition of 50 μl of a PBS solution (NaH_2PO_4, 2.7 m*M*; Na_2HPO4, 7.5 m*M*; NaCl, 150 m*M*; pH 7.4) containing the monoclonal anti-hIFN-α antibody (2:100 dilution), incubated overnight at 37 °C, and then washed with PBS. Samples were serially diluted in PBS-BSA-Tween buffer (BSA, 10 mg/ml; Tween-20, 2 mg/ml) and added to the wells. Following incubation at 37 °C for 1 h, the plates were washed with PBS. Next, 50 μl of PBS-BSA-Tween buffer containing rabbit anti-hIFN-α antibodies (1:1,000 dilution) was added. After 2 h incubation at room temperature, the plates were once again washed with PBS, and the bound antibody was detected by adding 50 μl of PBS-BSA-Tween buffer containing peroxidase-labeled goat anti-rabbit IgG (1:4,000 dilution). After 1 h incubation at room temperature, the plates were washed with PBS, and 100 μl of developing buffer (citric acid, 25 m*M*; Na_2HPO_4, 5 m*M*; *O*-phenylenediamine, 0.4 mg/ml; hydrogen peroxide, 0.012%; pH 5) was added. The enzymatic reaction was stopped after 10 – 15 min by the addition of 25 μl of 4 *N* sulfuric acid. The absorbance was read at 490 nm using a maximal volume (V_{max}) kinetic microplate reader, and the data were analyzed by the computer program SOFTMAX (Molecular Devices, Palo Alto, Calif.). Each assay included a standard curve of hIFN-α ranging from 10 to 1,250 IU/ml. The sensitivity limit of the assay was 20 IU/ml. The intra- and interassay coefficients of variation were 6% and 8%, respectively. The assay did not show cross-reactivity with murine serum or with murine peritoneal washings.

Results

Size distribution and efficiency of hIFN-α-2b encapsulation

Figure 1 shows the distribution of Depo/IFN as a function of the particle diameter. The average diameter of

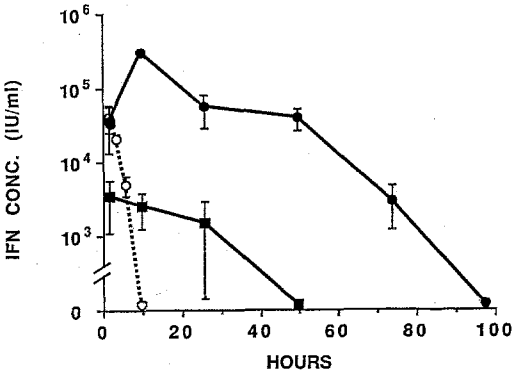

Fig. 3. Intraperitoneal concentrations of hIFN-α-2b after the administration of Std/IFN (*open circles*) and Depo/IFN (*filled circles*, total IFN; *filled squares*, free IFN). *Error bars*, SD. The final points of each pharmacokinetic curve were actual data points assayed to be below detectable limits

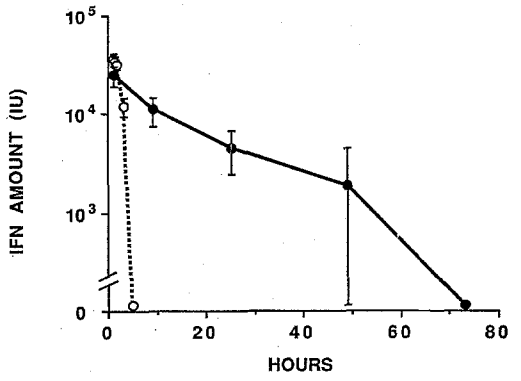

Fig. 4. Amount of hIFN-α-2b recovered from the peritoneal cavity after the administration of Std/IFN (*open circles*) and Depo/IFN (*filled circles*). *Error bars*, SD. The final points of each pharmacokinetic curve were actual data points assayed to be below detectable limits

Depo/IFN particles was 17.3 ± 5.6 μm (± SD of size distribution; $n = 3$). The capture efficiency was 51% ± 13% (± SD; $n = 3$) and the capture volume was 21 ± 5 μl/mg of total lipids used (± SD; $n = 3$).

In vitro release studies

In vitro plasma-release studies were done at 37 °C with human plasma. The release kinetics appeared to be of the first order (Fig. 2), with a half-life of 16 days (correlation coefficient, 0.998).

Intraperitoneal pharmacokinetics

After i.p. injection of Std/IFN, the protein was cleared from the peritoneal cavity with a half-life of 1.5 h (Fig. 3). Following injection of Depo/IFN, the total hIFN-α-2b concentration in the peritoneal cavity initially increased 8-fold during the first 8 h. Thereafter, the i.p. concentration declined slowly with a half-life of 20 h (Table 1). The free

Table 1. Intraperitoneal pharmacokinetic parameters

	Std/IFN	Depo/IFN	
		Free	Total
Concentration $t_{1/2}$ (h)[a]	1.5	20	20
Amount $t_{1/2}$ (h)[a]	1.3	NA	13
C_{max} ± SD (10^3 IU/ml)	30 ±3.3	2.9±2.0	250±7.7
AUC (10^3 IU ml^{-1} h)	82	85	4,900

C_{max}, Peak concentrations; AUC, area under the concentration-time curve; NA, not applicable

[a] Half-lives. All correlation coefficients for curve fitting were >0.98 except for the Depo/IFN total concentration (0.86)

hIFN-α-2b concentration (released from Depo/IFN) in the peritoneal cavity declined with an apparent half-life of 20 h. As shown in Fig. 4, the total absolute amount of hIFN-α-2b measured in the peritoneal cavity after the administration of Depo/IFN and Std/IFN largely paralleled that determined for concentrations. The AUC for free IFN was approximately the same after the administration of either Depo/IFN or Std/IFN.

Plasma pharmacokinetics

In plasma, hIFN-α-2b was undetectable for the Depo/IFN group (limit of detection, 20 IU/ml). For the Std/IFN group, the peak plasma concentration was 1,058 IU/ml at 2 h.

Discussion

In this paper, we report the encapsulation of hIFN-α-2b in a lipid-based drug-delivery system. The encapsulation efficiency (yield) and capture volume were reasonably high and were within usable ranges. The release rate in human plasma at 37 °C was slow, with a half-life of 16 days.

In vivo, the encapsulation of hIFN-α-2b increased the half-life of the total drug by a factor of 13, from 1.5 to 20 h. Following i.p. injection of Depo/IFN, the free hIFN-α-2b (released from Depo/IFN) peak concentration (C_{max}) was 10-fold lower and the apparent half-life was 13-fold higher, whereas the AUCs were similar to those determined following i.p. injection of Std/IFN.

The initial 8-fold increase in the peritoneal concentration of Depo/IFN was consistent with previous observations of encapsulated cytarabine [14] and methotrexate [6]. This rising concentration could be explained by a differential clearance of the suspending medium versus Depo/IFN particles.

A slow absorption of hIFN-α encapsulated in liposomes following intramuscular (i.m.) injection in mice has been reported [8 a, 8 b]. As assayed by inhibition of the cytopathic effect against vesicular stomatitis virus, the formulation gave almost quantitative retention at the injection site for up to 72 h after the injection. In comparison, unencapsulated interferon was undetectable after 24 h. A complete pharmacokinetic evaluation was not perform-

ed, as the encapsulation efficiency of this formulation was low (20%) and the stability in plasma at 37 °C was described only for the first 72 h of incubation [8 a].

More recently, Killion et al. [13] and Frangos et al. [9] reported enhanced antitumor activity for multilamellar liposomes containing hIFN-α against human bladder-tumor cell lines in vitro. The liposomes' stability was reported only for 4 h [9].

In humans, i.p. administration of hIFN-α alone or in combination with cisplatin has demonstrated activity in a subset of ovarian cancer patients with minimal residual disease (microscopic disease or tumor nodules measuring <5 mm in diameter) after first-line chemotherapy [1, 2, 16, 17, 25]. A recent study has confirmed the significant pharmacologic advantage for i.p. hIFN-α-2b, with the peritoneal fluid AUC being 30-fold higher than the systemic AUC [20]. Despite this observation, the maximum tolerated dose for i.p. hIFN-α ($25-50\times10^6$ IU/week) was limited by systemic absorption of the agent as reflected by the development of persistent general malaise, fever, gastrointestinal toxicity, and anemia [2, 16, 17, 25].

The results presented in this paper were obtained without extensive optimization of the formulation. It is likely that the encapsulation efficiency and the release characteristics can be improved further. We have not performed an in vivo efficacy study of Depo/IFN in rodents because of the species specificity of hIFN-α-2b. Such an in vivo efficacy test is needed to assure retention of the molecular and biological integrity of encapsulated hIFN-α-2b. However, we believe that the data presented in this paper justify further development of this formulation.

Acknowledgement. We thank Dr. Etienne Chatelut (Centre Claudius Regaud, Toulouse-France) for the kind assistance in analysis of the pharmacokinetic data.

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