Pharmacokinetics of Recombinant Human Interferon-β<sub>ser</sub> in Healthy Volunteers and Its Effect on Serum Neopterin

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Received June 9, 1992; accepted October 23, 1992

The pharmacokinetics of and biologic response modification by recombinant human interferon-β<sub>ser</sub> (rIFN-β<sub>ser</sub>) were evaluated in 12 healthy male volunteers. Subjects received a single intravenous (iv) injection of 90 × 10⁶ IU of rIFN-β<sub>ser</sub> followed by a single or eight consecutive daily 90 × 10⁶ IU subcutaneous (sc) doses. Blood samples collected after the iv, first sc, and last sc doses and prior to each sc dose were assayed for interferon antiviral activity and the interferon-inducible marker neopterin. Following iv administration, serum interferon concentrations generally declined biexponentially, with a mean serum clearance of 0.76 ± 0.28 L/hr·kg, a mean steady-state volume of distribution of 2.88 ± 1.81 L/kg, and a mean terminal half-life of 4.29 ± 2.29 hr as determined by noncompartmental analysis. Following sc administration, absorption of rIFN-β<sub>ser</sub> was prolonged, with serum concentrations generally below 100 IU/mL. No accumulation of rIFN-β<sub>ser</sub> in serum was noted after eight daily sc injections. In contrast, serum neopterin levels did not increase above baseline levels until 12 hr after iv dosing and 24 hr after sc dosing. The mean increase in serum neopterin at 24 hr post iv injection was significantly greater than that at 24 hr post sc dosing.

KEY WORDS: interferon-β; pharmacokinetics; biological response modification; neopterin.

INTRODUCTION

Recombinant human interferon-β<sub>ser</sub> (rIFN-β<sub>ser</sub>) is a genetically engineered, nonglycosylated recombinant human interferon beta which lacks the N-terminal methionine and has a serine residue substituted for the natural cysteine molecule at position 17 (1,2). rIFN-β<sub>ser</sub> has been shown to have broad-spectrum in vitro and in vivo antiviral and antiproliferative activities and in vitro immunomodulating properties as well as long-term safety and tolerance profiles in clinical trials (2–5).

Previous clinical pharmacokinetic studies have indicated that rIFN-β<sub>ser</sub> has a short terminal half-life after iv administration, and after sc administration rIFN-β<sub>ser</sub> serum concentrations are near or at the assay detection limit. In addition, rIFN-β<sub>ser</sub> as well as other lymphokines and cytokines, has been shown to induce a variety of biological responses in man (9–15). One of these markers, neopterin, is a by-product of GTP metabolism and has been associated with monocyte activation (11). In the present study, we investigated the pharmacokinetic characteristics of rIFN-β<sub>ser</sub> in conjunction with serum neopterin levels following iv and sc administration of rIFN-β<sub>ser</sub> to healthy volunteers, in order to aid in the optimization of treatment strategies.

MATERIALS AND METHODS

Subjects

Following selection and verification of eligibility, 12 normal healthy male volunteers ranging in age from 20 to 48 were entered into the study. All subjects gave informed consent prior to participation in the study. Physical examinations, EKG, urinalysis, hematology, and serum chemistry were conducted for all subjects.

Experimental Design

Recombinant human interferon-β<sub>ser</sub>, prepared as a lyophilized powder with a nominal specific activity of 1.8 × 10⁶ IU/mg, was supplied by Berlex Laboratories (formerly Triton Biosciences Inc.). Prior to injection, each vial of rIFN-β<sub>ser</sub> was reconstituted with 1.2 mL of 0.54% NaCl sterile solution, resulting in a concentration of 45 × 10⁶ IU/mL.

Each volunteer was scheduled to receive a single iv dose of rIFN-β<sub>ser</sub>, followed by a 1-week washout period and then eight consecutive daily sc doses of rIFN-β<sub>ser</sub>. The dose for both iv and sc administration was 90 × 10³ IU. An arm vein was used for iv bolus administration, while arms, abdomen, and thighs were used as sites of sc injection in a rotating manner. Each sc dose was divided into two 1-mL (45 × 10⁶ IU each) injections that were administered in rapid succession (within 2 min). Vital signs were measured within 30 min prior to dosing (predose) and at 30, 60, and 90 min after each iv and sc injection.

Blood samples were collected via arm vein after the iv dose, the first sc administration, and the final sc administration. Blood samples were also collected immediately prior to each sc dose. The arm opposite the one used for iv dosing was used for sample collection after iv administration. Sampling times after iv administration were 0 (predose), 5, 10, 15, 20, 30, 45, and 60 min and 2, 4, 6, 8, 12, and 24 hr postdose. After the first sc dose, the sampling times were 0 (predose), 30, 60, and 90 min and 2, 3, 4, 6, 8, 10, 12, and 24 hr after the dose. After the final sc injection, sampling times were the same as those after the first sc injection except that an additional sample was collected at 48 hr postdose. Serum collected from blood samples was immediately stored frozen at −70°C. All serum samples were assayed for serum interferon antiviral activity. Serum samples collected immediately before and after the iv and the first sc doses were assayed for serum neopterin levels.

Assay Methods

Serum interferon levels were quantitated using an assay which detects the inhibition of the cytopathic effects of vesicular stomatitis virus (VSV; Indiana strain) in the human
fibroblast cell line GM2504E (obtained from NIGMS Human Genetic Mutant Cell Repository, Camden, NJ), diploid for the class I human interferon receptor gene. Briefly, triplicate cell cultures were treated overnight with serial dilutions of serum samples, then challenged with VSV at a multiplicity of infection of 6 plaque-forming units/cell. After a minimum of 18 hr of incubation at 37°C, virus control cultures were microscopically examined to determine the level of viral CPE. Cultures treated with serum samples were examined, and a 50% CPE end point was determined relative to that of NIH interferon standards (Gxb02-901-535, human recombinant interferon beta; or Gb23-902-531, human native interferon beta). This assay had a detection limit of 20 IU/mL, with 13.3% variability at 1 IU/mL of the standard Gxb02-901-535.

The concentration of interferon in serum samples was defined as the reciprocal of the highest dilution of that sample that protected 50% of the cultured cells from viral CPE, relative to that of the standard interferon preparation.

Serum neopterin levels were determined using a commercially available radioimmunoassay kit (Neopterin-RIA, Henning Berlin GMBH, distributed by DRG International, Inc., Mountainside, NJ). Serum neopterin concentrations were determined by extraction from a neopterin concentration standard curve. Each serum sample was assayed in duplicate. The sensitivity of this assay was 0.9 pmol/mL. The intraassay and interassay coefficients of variation were 5.6 and 7.7%, respectively.

Data Analysis

Noncompartmental Analysis

After iv and sc administration, the peak serum interferon concentration (Cpeak) and time to peak (tpeak) were determined visually from individual serum concentration–time curves. Total area under the serum concentration–time curve (AUC) after iv administration of rIFN-βser was calculated using the linear trapezoidal rule from time 0 to the 8-hr time point and adding the value AUChr-inf where AUC8 hr-inf was calculated by dividing the concentration at 8 hr postdose by the terminal rate constant (β). Total area under the first moment serum concentration–time curve (AUMC) after iv injection was calculated using the linear trapezoidal rule on the concentration × time versus time curve from time 0 to the 8-hr time point and then adding the sum of the following two terms: (C8 hr × 8 hr/β) + (C8 hr/β²), where C8 hr is the serum interferon concentration 8 hr postdose.

Serum clearance (CL) after iv bolus administration was calculated by dividing the dose by the AUC. Volume of distribution at steady state (Vss) after an iv bolus dose was calculated as dose times AUMC divided by AUC. Terminal half-life (t1/2) was computed by linear regression performed on the terminal phase of the log-linear serum concentration–time curve. Mean residence time (MRT) of rIFN-βser after iv bolus administration was calculated by dividing AUMC by AUC.

Total AUC after single and multiple sc doses was calculated using the linear trapezoidal rule from time 0 to 24 hr postdose and adding AUC from 24 hr postdose to infinity (AUC24 hr-inf). Samples with interferon concentrations at or below the detection limit of the assay were assumed to contain 20 IU/mL of interferon. It was also assumed that the absorption process was complete by 24 hr after sc dosing and that the apparent terminal rate constant for elimination of rIFN-βser from serum was the same for both iv and sc administration in a given subject. The iv terminal rate constant was therefore used to determine the AUC24 hr-inf for sc dosing.

AUC24 hr-inf was calculated by dividing the serum interferon concentration at 24 hr postdose by the terminal rate constant (β) obtained for that subject after iv administration. Bioavailability (F) after a single sc administration was calculated as AUC after a single sc dose divided by AUC after iv dosing of rIFN-βser in the same subject. The accumulation factor after multiple sc doses was calculated as AUC after multiple sc doses divided by AUC after a single sc dose in the same subject.

Compartmental Analysis

After a single iv dose of rIFN-βser, serum concentration data were also evaluated using compartmental analysis. Concentration–time data were fitted to a two-compartment model function \( C = A \times e^{-at} + B \times e^{-bt} \) using PCNONLIN program version 3.0 (SCI, Lexington, KY), where C is the serum interferon concentration at time t, A and B are the extrapolated initial concentrations for the two compartments, and a and b are the macro-elimination rate constants for the two compartments.

In evaluating the pharmacokinetic data for the 12 subjects after iv administration, the concentration–time profiles for subjects 1, 4, and 5 resembled those observed after sc dosing with serum concentration data either near or at the assay detection limit, suggesting that rIFN-βser was not administered iv. Therefore, iv data collected from these three subjects, including Cpeak and tpeak, were excluded from analysis. In addition, the AUC values after sc administration for subject 1, 4, and 5 were not calculated due to lack of IV pharmacokinetic information necessary to determine terminal t1/2.

Statistical Analysis

The Cpeak and AUC values obtained after single and repeated sc dosing were compared using the paired t test. Paired differences in neopterin levels after single iv and single sc doses were calculated and the distribution of these differences was determined to be approximately normal. One-way analysis of variance models with assay run as a factor were used to test hypotheses that the mean paired differences were equal to zero. A 5% two-tailed significance level was used for all statistical hypothesis tests.

RESULTS

Demographics, Safety, and Tolerance of rIFN-βser

The twelve subjects enrolled in the study ranged in age between 20 and 48 (mean, 32 ± 9), measured between 68 and 77 in. in height (mean, 72 ± 3 in.) and weighed between 144 and 212 lbs (mean, 178 ± 22 lb) at screen.

At screen, the physical examination, EKG, urinalysis, hematology, and serum chemistry for all subjects were within normal ranges. Following rIFN-βser administration, all 12 subjects developed mild to moderate side effects, in-