Pharmacokinetics of Recombinant Human Interferon-β_{ser} in Healthy Volunteers and Its Effect on Serum Neopterin

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The pharmacokinetics of and biologic response modification by recombinant human interferon-β_{ser} (rIFN-β_{ser}) were evaluated in 12 healthy male volunteers. Subjects received a single intravenous (iv) injection of 90×10^6 IU of rIFN-β_{ser} followed by a single or eight consecutive daily 90×10^6 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_{ser} was prolonged, with serum concentrations generally below 100 IU/mL. No accumulation of rIFN-β_{ser} 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_{ser} (rIFN-β_{ser}) 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_{ser} 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_{ser} has a short terminal half-life after iv administration, and after sc administration rIFN-β_{ser} serum concentrations are near or at the assay detection limit. In addition, rIFN-β_{ser}, as well as other lymphokines and cytokines, has been shown to induce a variety of biological response markers 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_{ser} in conjunction with serum neopterin levels following iv and sc administration of rIFN-β_{ser} 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_{ser}, prepared as a lyophilized powder with a nominal specific activity of 1.8×10^8 IU/mg, was supplied by Berlex Laboratories (formerly Triton Biosciences Inc.). Prior to injection, each vial of rIFN-β_{ser} was reconstituted with 1.2 mL of 0.54% NaCl sterile solution, resulting in a concentration of 45×10^6 IU/mL.

Each volunteer was scheduled to receive a single iv dose of rIFN-β_{ser}, followed by a 1-week washout period and then eight consecutive daily sc doses of rIFN-β_{ser}. The dose for both iv and sc administration was 90×10^6 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 (45×10^6 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

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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 reciprocol 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 extrapolation 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 (C_{peak}) and time to peak (t_{peak}) were determined visually from individual serum concentration–time curves. Total area under the serum concentration–time curve (AUC) after iv administration of rIFN-β_{ser} was calculated using the linear trapezoidal rule from time 0 to the 8-hr time point and adding the value $AUC_{8\ hr\text{-}inf}$, where $AUC_{8\ hr\text{-}inf}$ 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: $(C_{8\ hr} \times 8\ hr/\beta) + (C_{8\ hr}/\beta^2)$, where $C_{8\ hr}$ 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 (V_{ss}) after an iv bolus dose was calculated as dose times AUMC divided by AUC^2. Terminal half-life ($t_{1/2}$) was computed by linear regression performed on the terminal phase of the log-linear serum concentration–time curve. Mean residence time (MRT) of rIFN-β_{ser} 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 ($AUC_{24\ hr\text{-}inf}$). 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-β_{ser} 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 $AUC_{24\ hr\text{-}inf}$ for sc dosing.

$AUC_{24\ hr\text{-}inf}$ 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-β_{ser} 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-β_{ser}, serum concentration data were also evaluated using compartmental analysis. Concentration–time data were fitted to a two-compartment model function [$C = A \times e^{(-\alpha t)} + B \times e^{(-\beta t)}$] 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 α and β 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-β_{ser} was not administered iv. Therefore, iv data collected from these three subjects, including C_{peak} and t_{peak}, 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 $t_{1/2}$.

Statistical Analysis

The C_{peak} 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-β_{ser}

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-β_{ser} administration, all 12 subjects developed mild to moderate side effects, in-

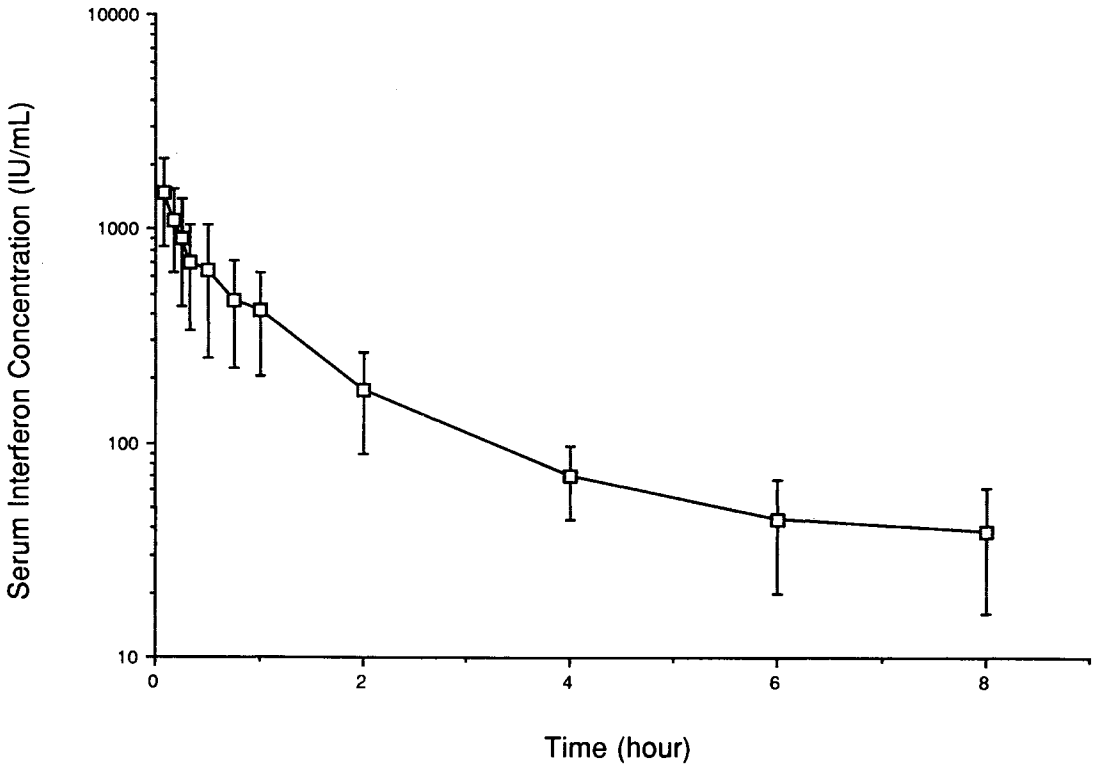

Fig. 1. Mean serum interferon concentration–time profile following a single iv administration of 90 × 10^6 IU rIFN-β_{ser}. Values are for nine subjects, with vertical bars indicating standard deviation.

cluding headaches, chills, fever, sweating, nausea, vomiting, body aches, joint aches, pain or rash at injection site, flatulence, and arthralgia. Because of these side effects, subjects 1, 2, 3, 5, 7, and 10 chose to withdraw from the study after serum samples were collected following the iv dose and the first sc dose. The other six subjects (Nos. 4, 6, 8, 9, 11, and 12) completed the study. For the six subjects who completed the study, body weight changes did not exceed 5% of initial body weight.

Pharmacokinetics of rIFN-β_{ser}

Following a single iv dose of rIFN-β_{ser}, mean peak serum interferon concentrations occurred at the earliest sampling time, 5 min postdose (Fig. 1). Serum interferon concentrations for the 12- and 24-hr sampling times were either at or near the assay detection limit (20 IU/mL). For this reason all data points obtained more than 8 hr after iv administration were not utilized in evaluating the pharmacokinetics of rIFN-β_{ser}.

Compartmental and noncompartmental pharmacokinetic analysis were performed on data obtained for each subject after iv administration of rIFN-β_{ser}. The mean pharmacokinetic parameters obtained by these two analyses were generally comparable (Tables I and II).

Following single and repeated sc administration of 90 × 10^6 IU rIFN-β_{ser}, serum interferon concentrations were generally below 100 IU/mL, with approximately one-half of the

Table I. Pharmacokinetic Parameters Following a Single iv Administration of 90 × 10^6 IU rIFN-β_{ser}: Noncompartmental Analysis (n = 9)

Subject No.	C_{peak} (IU/mL)	AUC (IU-hr/mL)	$t_{1/2}$ (hr)	CL (L/hr-kg)	V_{ss} (L/kg)	MRT (hr)
2	998	975	2.88	1.07	3.02	2.81
3	998	1995	3.59	0.49	1.90	3.91
6	2112	1099	1.71	1.26	2.07	1.64
7	1877	2660	4.54	0.47	1.33	2.84
8	1877	2481	4.05	0.47	1.64	3.48
9	2347	2325	6.29	0.59	3.63	6.19
10	1877	1514	1.98	0.74	1.31	1.76
11	499	1179	9.13	0.80	6.88	8.65
12	832	1117	4.48	0.91	4.15	4.58
Mean	1491	1705	4.29	0.76	2.88	3.98
SD	±659	±665	±2.29	±0.28	±1.81	±2.25

Table II. Mean Pharmacokinetic Parameters Following a Single iv Administration of 90 × 10^6 IU rIFN-β_{ser}: Compartmental Analysis (n = 9)

	A (IU/mL)	B (IU/mL)	α (hr^{-1})	β (hr^{-1})	AUC (IU-hr/mL)	$t_{1/2,\alpha}$ (hr)	$t_{1/2,\beta}$ (hr)	CL (L/hr-kg)	V_c (L/kg)	V_{ss} (L/kg)
Mean	1675	221	3.33	0.249	1688	0.42	4.35	0.77	0.83	2.62
SD	±1198	±156	±3.49	±0.182	±686	±0.30	±2.77	±0.28	±0.47	±1.37

serum samples collected between 30 min and 24 hr postdose having interferon concentrations near or at the assay sensitivity limit (Fig. 2 and data now shown). A prolonged absorption process was noted following SC administration of 90 × 10^6 IU rIFN-β_{ser}, with t_{peak} occurring 1–8 hr after sc administration. The average peak serum concentration after a single sc dose was 40 ± 20 IU/mL and 44 ± 12 IU/mL after repeated dosing (Table III). There was no statistically significant difference between the two C_{peak} values (P = 0.69).

Neopterin Induction by rIFN-β_{ser}

Serum neopterin levels after rIFN-β_{ser} administration are presented in Table IV. After a single iv injection of 90 × 10^6 of rIFN-β_{ser}, mean serum neopterin concentrations were not significantly greater than baseline levels until 12 hr postinjection (P = 0.0001). Mean serum neopterin levels doubled between 12 and 24 hr after dosing, with mean values significantly greater at 24 hr than at 12 hr (P = 0.0001). Serum neopterin levels returned to baseline levels by 7 days after iv rIFN-β_{ser} dosing (P = 0.90, comparison between serum neopterin levels at 0 hr iv and 7 days postinjection).

In contrast, serum neopterin concentrations after sc administration were not significantly increased above baseline levels until 24 hr postinjection (P = 0.0001). The magnitude of neopterin increase between baseline and 24 hr after iv administration of rIFN-β_{ser} was significantly greater than that measured over the same time interval for sc injection (P = 0.0001).

DISCUSSION

This study demonstrated that following iv administration of rIFN-β_{ser} to healthy volunteers, serum interferon concentrations generally showed a biexponential decline, with a terminal $t_{1/2}$ of approximately 4 hr. This finding is comparable to reports in the literature, where the rIFN-β_{ser} concentration–time curve showed a biexponential decay with rIFN-β_{ser} having a terminal $t_{1/2}$ of 30 to 103 min (6–8). Following single and repeated sc dosing, serum interferon concentrations were found to be generally low and below 100 IU/mL, consistent with published information (9).

Mean AUC values were estimated to be 796 ± 230 and 927 ± 355 IU-hr/mL after single and repeated sc doses, respectively (Table III), with no statistically significant difference between these two AUC values (P = 0.93). The mean accumulation factor after repeated sc dosing was 0.97, indicating no apparent accumulation of rIFN-β_{ser} with daily sc

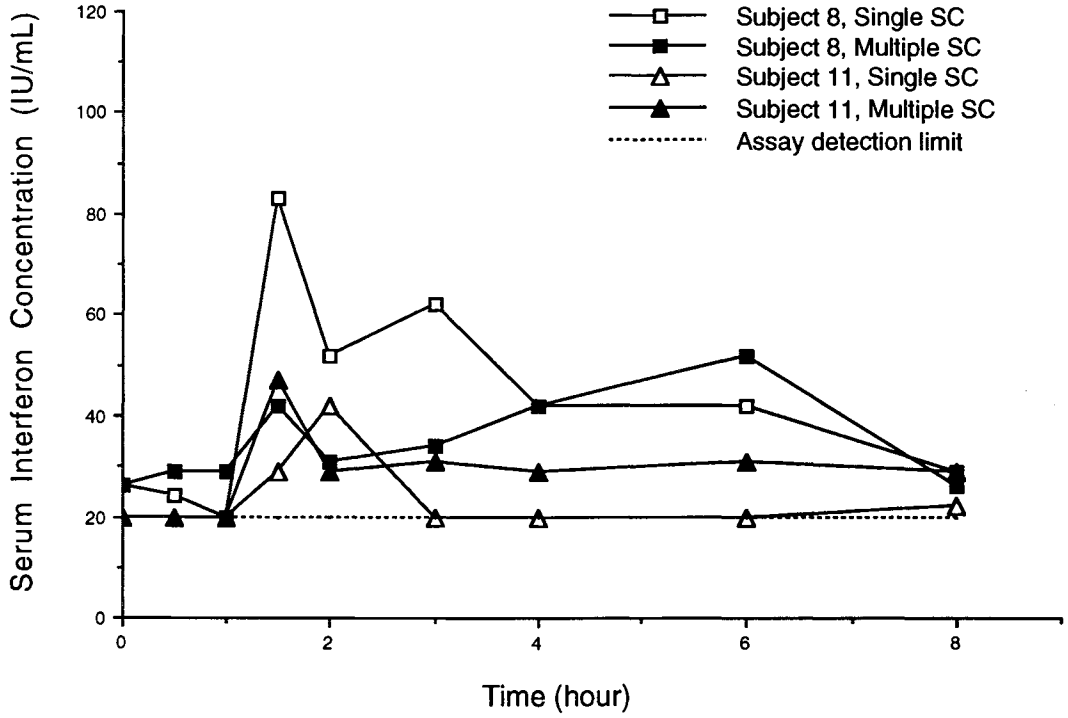

Fig. 2. Serum interferon concentration–time profiles following single and multiple sc doses of 90 × 10^6 IU rIFN-β_{ser} to two representative subjects.

Table III. Mean Pharmacokinetic Parameters Following Single and Multiple sc Administrations of 90×10^6 IU rIFN-β_{ser}

	Single dose		Multiple doses		
	C_{peak} (IU/mL)	AUC (IU-hr/mL)	C_{peak} (IU/mL)	AUC (IU-hr/mL)	Accumulation factor
Mean	40	796	44	927	0.97
SD	±20	±230	±12	±355	±0.14
n	12	9[a]	6[b]	5[c]	5[c]

[a] The iv data from three subjects were excluded from pharmacokinetic analysis.
[b] Six subjects withdrew from the study.
[c] Six subjects withdrew from the study and the iv data from one subject were excluded from pharmacokinetic analysis.

dosing for 8 days. In addition, low serum interferon concentrations in samples obtained prior to the third through eighth sc doses also suggested no accumulation of rIFN-β_{ser} (data not shown). Mean bioavailability (*F*), after two sc injections totaling 90×10^6 IU, was approximately 51 ± 17%. However, the AUC and bioavailability values determined after sc administration may be overestimated due to the assumption made regarding serum interferon concentrations at the limit of assay sensitivity and the assumption that the terminal rate constant, obtained from iv dosing data to define serum interferon levels beginning 24 hr after sc administration, may lead to an underestimate of the sc values. Both assumptions, though reasonable, should be noted when interpreting these results.

This study also demonstrates that despite low serum levels of interferon after sc administration of rIFN-β_{ser}, serum neopterin levels are significantly increased after both iv and sc treatments. This finding supplements previous reports that neopterin levels are elevated in subjects administered rIFN-β_{ser}, interferon-alpha, interferon-gamma, tumor necrosis factor, or interleukin-2 (9,15–17). Although serum interferon concentrations peaked at 5 min after iv injection and at 1–8 hr after sc administration of rIFN-β_{ser}, serum neopterin levels did not significantly increase above baseline values until 12 hr after iv dosing and 24 hr after sc dosing. In addition, serum neopterin levels at 24 hr after sc dosing were significantly lower than those at 24 hr post iv dosing. Similar findings have been reported by Goldstein *et al.* (9), where significantly greater increases in serum neopterin levels at 24 and 48 hr after iv dosing were observed than at 24 and 48 hr after sc dosing. The smaller increase in neopterin levels after sc administration may be associated with the prolonged absorption and/or lower bioavailability of rIFN-β_{ser} after SC administration. However, no correlation between AUC and the difference between baseline and 24 hr postdose serum neopterin levels was observed for the iv (Spearman, 0.22) and sc (Spearman, −0.27) dose groups. Other investigators have reported that rIFN-β_{ser} induces greater increases in the levels of other biological response markers, including 2′,5′-oligoadenylate synthetase and natural killer cell activity, after sc administration than after iv administration of the same dose (9). These results suggest that, depending on the route of administration of rIFN-β_{ser}, it may be more appropriate to measure the changes in the levels of one or more specific biologic response markers rather than others in order to assess the overall biologic response to rIFN-β_{ser} treatment.

In conclusion, results from this study, combined with prior observations, further define the relationship between the rIFN-β_{ser} pharmacokinetic profile and the induction of the biologic response to rIFN-β_{ser} treatment and may serve as a useful tool in determining a dose and regimen of rIFN-β_{ser} resulting in an optimal biologic response.

Table IV. Mean Serum Neopterin Concentrations Following iv and sc Administration of 90×10^6 IU rIFN-β_{ser} (Mean ± SD; $n = 12$)

Time since injection (hr)	Following iv administration (n*M*)	Following sc administration (n*M*)
0	6.56 ± 4.93	5.73 ± 1.33
2	5.53 ± 3.81	5.20 ± 1.31
4	5.44 ± 4.01	4.84 ± 1.14
6	6.72 ± 4.34	5.06 ± 1.36
8	6.51 ± 3.08	4.48 ± 0.97
12	12.33 ± 4.36[a]	6.25 ± 1.50
24	28.38 ± 6.60[b]	17.02 ± 2.79[c]

[a] Significantly greater than baseline value prior to iv dosing.
[b] Significantly greater than baseline value prior to iv dosing and value at 12 hr post iv dosing.
[c] Significantly greater than baseline value prior to sc dosing and significantly different from value at 24 hr post iv dosing.

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