recombinant human interferon beta-1a (IFN-β-1a) forumulation

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INTRODUCTION

Interferons are a family of proteins that have antiviral activity, inhibit cell proliferation, and modulate the natural immune response (1). Human interferon beta (IFN-β), a member of this family, is a 166 amino acid glycoprotein produced by fibroblasts, as well as other cells, after induction by viral infection or by double-stranded RNA. Over the past 20 years, the following three forms of IFN-β have been clinically tested in a wide variety of human disorders (2): natural human IFN-β produced from human foreskin fibroblasts (n-IFN-β), recombinant human IFN-β produced in *E.Coli* (IFN-β-1b, containing a genetically engineered serine substitution for cystine at position 17), and recombinant human IFN-β produced in Chinese hamster ovary cells (IFN-β-1a, containing the natural human amino acid sequence). Both n-IFN-β and IFN-β-1a are glycosylated with a single N-linked complex carbohydrate moiety whereas IFN-β-1b is not glycosylated.

AVONEX™, one IFN-β-1a product, demonstrated an effect of treatment in slowing the accumulation of physical disability and decreasing the frequency of clinical exacerbations in patients with relapsing multiple sclerosis when administered at a once-weekly intramuscular (IM)

dose of 6 million units (MU) (3). A second IFN-β-1a product, Rebif®, is being given subcutaneously (SC) in ongoing phase III trials in the treatment of multiple sclerosis. Previous studies with AVONEX™ and Rebif® had raised concerns that while these products are both designated as IFN-β-1a, they may not have similar pharmacokinetic and subsequent pharmacodynamic profiles (4-7).

In order to determine whether AVONEX™ and Rebif® may be used interchangeably, a pharmacokinetic and pharmacodynamic intra-subject crossover comparison of the two products after IM injection in healthy male and female volunteers was conducted. The results reported here demonstrate the surprising finding that AVONEX™ and Rebif® are not equivalent when administered intramuscularly. AVONEX™ is formulated in a higher concentration of albumin (15 mg/mL after reconstitution *versus* 9 mg/mL for Rebif®), at a different pH (7.2 versus 5.5) and in a different buffer (phosphate *versus* acetate) (5,7,15,16). In addition, Rebif® contains mannitol in its formulation and AVONEX™ does not. These formulation differences may contribute to the unexpected altered absorption of IFN-β after IM injection by affecting binding to the muscle extracellular matrix and/or inactivation by pH-dependent proteases (17)

To further evaluate the effect of IM injection on these properties, a further aim of this study was to assess the pharmacokinetics and pharmacodynamics of one specific formulation of IFN-β-1a, Avonex™, after intravenous (IV), subcutaneous (SC) or intramuscular (IM) administration in healthy volunteers. Three groups, each consisting of 8 healthy male and female subjects, received a single 60 μg (12 million units) dose of IFN-β-1a by either a 30 minute intravenous (IV) infusion, or by subcutaneous (SC) or intramuscular (IM) injection. We show that serum interferon activity levels peaked immediately after the end of the IV infusion and thereafter fit a biexponential decay model. Interferon activity was consistently detectable in serum after IM injection but not after SC injection. The pharmacodynamic response, as measured by changes serum neopterin and β_2-microglobulin concentrations, was also greatest after IM injection, followed by SC, then IV administration. These results indicate that the optimal pharmacokinetic and pharmacodynamic response is produced by IM, rather than SC or IV administration of IFN-β-1a.

Preferred embodiments of the invention for IM administration include a packaged kit for

parenteral administration of the present liquid formulations. The package may include syringes pre-filled with the liquid formulations of the invention, several alcohol swabs, at least one needle, one or more adhesive bandages and directions for use. It will also appreciated that the present liquid formulations of the invention may be used with conventional needleless- injection systems.

METHODS

Study Design

A. Avonex v. Rebif

This was a randomized, open-label, single-center, two-treatment, crossover study designed to compare the pharmacokinetic and pharmacodynamic profiles of AVONEX™ and Rebif® after IM injection in healthy volunteers. Male and female subjects, aged between 18 and 45 years, who were within 15% of normal body weight for height with a minimum body weight of 50 kg, and who gave written informed consent, were eligible for this study.

Fifteen male and 15 female subjects were enrolled. Subjects were to receive by IM injection one 6 MU dose of AVONEX™ and one 6 MU dose of Rebif® with doses administered 2 weeks apart. All but one subject completed the study. A female who received Rebif® in the first study period developed headaches and pharyngitis; she withdrew prior to the AVONEX™ injection. This subject was not included in any of the pharmacokinetic or pharmacodynamic analyses. Intramuscular injection was to the anterolateral aspect of the thigh. Doses were administered at equivalent contralateral injection sites, i.e., AVONEX™ and Rebif® were not given at the same injection site.

Subjects entered a clinical pharmacology unit within 24 hours prior to each injection and remained in the unit until the completion of the evaluations at 24 hours post-dosing. Subjects were confined to bed for one hour following each injection; thereafter, normal activity was allowed.

Subjects received paracetamol (acetaminophen) following each injection to reduce the likelihood of flu-like symptoms associated with interferon administration. Subjects were instructed not to take any other medication for the duration of study participation.

Blood samples for pharmacokinetic determinations were collected immediately prior to each dose and at 6, 9, 12, 15, 18, 24, 30, 36, and 48 hours post-dose. Pharmacodynamics were assessed by evaluation of serum neopterin concentration profiles. Neopterin is a product of interferon-induced GTP cyclohydrolase; elevations in serum neopterin post-injection reflect a downstream biologic response to receptor activation by IFN-β (8,9). Blood for determination of serum neopterin concentration was collected immediately prior to each dose, and at 6, 12, 18, 24, 30, 36, 48, 72, 96, and 144 hours following each dose.

Adverse events were recorded throughout the study. Routine hematology and blood chemistry tests were performed 144 hours after each injection. This study was conducted in the United Kingdom and adhered to the tenets of the Declaration of Helsinki.

B. Single Dose Study

This was a single dose, parallel group study to evaluate the comparative pharmacokinetics and pharmacodynamics of IFN-β-1a by three different routes of administration: SC, IM and 30 minute IV infusion.

Healthy males and females, aged between 18 to 45, who were within 15% of normal body weight for height with a minimum body weight of 50 kg, and who gave written informed consent, were eligible for this study. Subjects were deemed healthy upon completion of the following: a physical examination; an electrocardiogram (ECG); and blood and urine laboratory testing that included tests for Hepatitis B antigen, Hepatitis C, and HIV. Female subjects who were pregnant or breast-feeding were excluded.

Twenty-four subjects (12 males and 12 females) were enrolled and assigned to groups of eight to receive 60 μg of IFN-β-1a by either SC or IM injection, or as an IV infusion over 30 minutes. Equal numbers of males and females were entered into each group.

Blood was drawn for pharmacokinetic testing at 1, 3, 6, 9, 12, 15, 18, 24, and 48 hours following each SC or IM injection, and at 10, 20, and 30 minutes following the start of the IV infusion and at 5, 10, 20, 30, 45, 60, and 90 minutes, and at 2, 3, 4, 5, 6, 9, 12, 15, 18, 24, and 48 hours following the end of the IV infusion.

Measurement of two interferon induced biological response markers, serum neopterin and

β_2-microglobulin, (9) were performed at 6, 12, 24, and 48 hours following administration of study drug.

Urinalysis, blood chemistry, and hematology tests were performed at 48 hours following study drug. Adverse events were monitored throughout the study period.

The study was performed at a clinical pharmacology unit in the United Kingdom after local ethical committee approval.

Study Drug

A. Avonex v. Rebif Comparison

AVONEX™ was supplied as a sterile lyophilized powder containing IFN-β-1a, HSA, sodium phosphate, and sodium chloride; prior to injection, the vial contents were reconstituted with Sterile Water for Injection. Rebif® was available as a sterile lyophilized powder containing IFN-β-1a, mannitol, HSA, and sodium acetate. Rebif® was reconstituted with sodium chloride solution (0.9% NaCl) for injection. Each product was stored as a lyophilized powder at 2-8°C prior to reconstitution. AVONEX™ was packaged in 6 MU vials and Rebif® was packaged in 3 MU vials. The activity of each was accepted as stated on the label.

Each vial of Rebif® was reconstituted as directed using 1 mL of the supplied vehicle. Two reconstituted vials were combined to prepare a 6 MU dose in a volume of approximately 2 mL. Each vial of AVONEX™ was also reconstituted as directed to prepare a 1 mL solution. However, in order to inject equal volumes of the two test drugs, matching placebo vials containing excipient only were also reconstituted with 1 mL of sterile water. One reconstituted vial each of AVONEX™ and placebo were then combined to prepare a 6 MU injection in a volume of 2 mL.

B. Single Dose Study

IFN-β-1a (Avonex™) was supplied as a lyophilized powder in vials containing 60 μg of IFN-β-1a, as well as human serum albumin, sodium chloride and sodium phosphate. Each vial was reconstituted with 1 mL of sterile water prior to injection. The specific activity of the IFN-β-1a used in this study was 200 million units (MU) of antiviral (interferon) activity per milligram of IFN-β-1a protein. Thus, each vial contained 12 MU of interferon activity.

Assay Methods

A. Avonex v. Rebif Comparison

Levels of IFN-β in serum were quantitated at Biogen using a cytopathic effect (CPE) bioassay. The CPE assay measured levels of interferon-mediated antiviral activity which were proportional to the concentration of active interferon the serum sample. Historically, this assay has been the standard method to assess the pharmacokinetics of IFN-β (10,11).

The CPE assay detected the ability of interferon beta (IFN-β) to protect human lung carcinoma cells (A549, #CCL-185, ATCC, Rockville, MD) from cytotoxicity due to encephalomyelocarditis (EMC) virus. The cells were preincubated for 15 to 20 hours with serum samples to allow the induction and synthesis of interferon inducible proteins that produce an antiviral response. Following pre-incubation, EMC virus was added to each well and incubated for an additional 30 hours; cytotoxicity was determined using a crystal violet stain. An internal Biogen IFN-β standard was tested concurrently with samples on each assay plate. This standard has been calibrated against a natural human fibroblast interferon reference standard (WHO Second International Standard for Interferon, Human Fibroblast, Gb-23-902-531) (12).

Serum samples and standards were tested in duplicate on each of two replicate assay plates, yielding four data points per sample. The geometric mean concentration of the four replicates was reported.

The inter-assay variability was determined by calculating the 95% confidence interval about the mean internal IFN-β standard concentration for 323 assay plates. As defined, variability was less than 10% of the mean. The limit of quantitation was generally 10 U/mL. Serum neopterin concentrations were determined using a commercially available ^{125}I RIA kit (Immuno Biological Laboratories, Hamburg, Germany). Study personnel performing pharmacokinetic and pharmacodynamic assays were blinded to treatment assignment.

B. Single Dose Study

Levels of IFN-β in serum were quantitated at Biogen using a cytopathic effect (CPE) bioassay. The CPE assay measures levels of interferon-mediated antiviral activity. The level of antiviral activity in a sample reflects the number of molecules of active interferon contained in

that sample at the time the blood was drawn. This approach has been the standard method to assess the pharmacokinetics of IFN-β (11).

The CPE assay used in the current study detects the ability of interferon beta (IFN-β) to protect human lung carcinoma cells (A549, #CCL-185, ATCC, Rockville, MD) from cytotoxicity due to encephalomyelocarditis (EMC) virus. The cells are preincubated for 15 to 20 hours with serum samples to allow the induction and synthesis of interferon inducible proteins that then mount an antiviral response. Afterwards EMC virus is added and incubated for a further 30 hours before assessment of cytotoxicity is made using a crystal violet stain. An internal Biogen interferon beta standard is tested concurrently with samples on each assay plate. This standard has been calibrated against a natural human fibroblast interferon reference standard (WHO Second International Standard for Interferon, Human Fibroblast, Gb-23-902-53 (12). Each assay plate also includes cell growth control wells containing neither interferon beta nor EMC, and virus control wells containing cells and EMC but no interferon beta. Control plates containing the standard and samples are also prepared to determine the effect, if any, of the samples on cell growth. These plates are stained without the addition of virus.

Samples and standards are tested in duplicate on each of two replicate assay plates, yielding four data points per sample. The geometric mean concentration of the four replicates is reported. The limit of detection in this assay is 10 units (U)/mL.

Serum concentrations of neopterin and β_2-microglobulin were determined at the clinical pharmacology unit using commercially available assays. Neopterin determinations were not performed for the IV dosing group due to unavailability of the assay from the manufacturer at the time this group was enrolled.

Pharmacokinetic and Statistical Methods

A. Avonex v. Rebif Comparison

Standard descriptive analysis of serum interferon activity data for both IFN-β-1a products was conducted. The following pharmacokinetic parameters were calculated:

(i) area under the curve, AUC (U×h/mL), from 0 to 48 hours post dosing, using the trapezoidal algorithm;

(ii) observed peak serum activity, C_{max} (U/mL), by inspection; and

(iii) time to peak serum activity, t_{max} (h), by inspection.

In the calculation of AUC and C_{max}, the baseline serum interferon activity was subtracted from all post-dose values; all undetectable values were set to 0 U/mL. However, in the calculation of relative bioavailability, undetectable post-dosing values were set to 5 U/mL (which represented half of the lower limit of quantitation). This was done because serum interferon activity levels were 0 U/mL throughout the period after Rebif® administration in five subjects and after AVONEX™ in one subject. In the case of the five Rebif® subjects, adjusting nondetectable concentrations to 5 U/mL permitted the calculation of a finite relative bioavailability.

AUC and C_{max} were analyzed using a two-way crossover analysis of variance (ANOVA). Terms in the analysis included sequence, subject, gender, period, and treatment (13). A term for the gender-by-treatment interaction was initially included, but subsequently removed because the interaction was not significant . AUC and C_{max} were logarithmically transformed prior to analysis.

The following pharmacodynamic parameters were calculated:

(i) area under the curve normalized to baseline, from 0 through 144 hours, E_{AUC};

(ii) maximum increase from baseline, E_{max};

(iii) time, t_{max}, at which the maximum effect was noted; and

(iv) concentration difference between 0 and 144 hours after dosing.

All E_{AUC} and E_{max} values were baseline corrected. Statistical analysis was performed on $\log_e E_{AUC}$ and $\log_e E_{max}$ using the two-way crossover ANOVA described above. Estimates of the ratios of E_{AUC} and E_{max} of AVONEX™ to Rebif® with 90% confidence limits were also made. To determine if serum neopterin concentrations at 144 hours post-dose were still elevated following administration of each product, the differences between the baseline and 144-hour value were compared with zero using a paired t-test.

B. Single Dose Study

Rstrip® software (MicroMath, Inc., Salt Lake City, UT) was used to fit data to

pharmacokinetic models. Geometric mean concentrations were plotted by time for each group. Since assay results are expressed in dilutions, geometric means are considered more appropriate than arithmetic means. Serum interferon levels were adjusted for baseline values and non-detectable serum concentrations were set to 5 U/mL, which represents one-half the lower limit of detection.

For the IV infusion data, a two compartment IV infusion model was fit to the detectable serum concentrations for each subject, and the SC and IM data were fit to a two compartment injection model.

The following pharmacokinetic parameters (20) were calculated:

(i) observed peak concentration, C_{max} (U/mL);

(ii) area under the curve from 0 to 48 hours, AUC (U·h/mL) using the trapezoidal rule;

(iii) elimination half-life;

and, additionally from IV infusion data:

(iv) distribution half-life (h);

(v) clearance (mL/h)

(vi) apparent volume of distribution, Vd (L).

WinNonlin (Version 1.0, Scientific Consulting Inc., Apex, NC) software was used to calculate the elimination half-lives after SC and IM injection.

For β_2-microglobulin and neopterin, arithmetic means by time have been presented for each group. E_{max}, the maximum change from baseline, was calculated. C_{max}, AUC and E_{max} were submitted to a one-way analysis of variance to compare dosing groups. C_{max} and AUC were logarithmically transformed prior to analysis; geometric means are reported.

RESULTS AND DISCUSSION

A. Avonex v. Rebif Comparison

Figure 1 displays the mean serum interferon activity by time for each product. At each post-dose timepoint, mean serum interferon activity following AVONEX™ administration was higher than that following Rebif® administration. Table I summarizes the pharmacokinetic

parameters for each product and the results from the crossover analyses of variance. The least squares mean AUC values for AVONEX™ and Rebif® were 824 and 403 U×h/mL, respectively. The least squares mean ratio of AUC for AVONEX™ to Rebif® was 204% with 90% confidence limits of 172 to 243% ($p<0.001$).

Least squares mean C_{max} values were 33.8 U/mL following AVONEX™ administration and 15.2 U/mL following Rebif® administration. The least squares mean ratio of C_{max} for AVONEX™ to Rebif® was 222% with 90% confidence limits of 172 to 285% ($p<0.001$). The mean time to maximum concentration was between 12 and 16 hours for AVONEX™ and Rebif®.

The pharmacodynamic effect of AVONEX™ paralleled the pharmocokinetic findings. Figure 2 illustrates the drug-related geometric mean neopterin concentrations versus time following each treatment. Following either product, neopterin concentrations rose during the initial 36 hours; concentrations plateaued from 36 through 72 hours post-dose and then gradually declined. However, neopterin induction was greater for AVONEX™ as compared to Rebif®. Mean concentrations during the 36 to 72 hours post dose time period were approximately 12.0 nmol/L for AVONEX™ and 9.3 nmol/L for Rebif®. Neopterin concentrations at 144 hours post-dose were significantly higher than pre-dose for each treatment ($p<0.001$).

Table II summarizes the pharmacodynamic parameters, E_{AUC} and E_{max}. The least squares mean E_{AUC} values for AVONEX™ and Rebif® were 693 and 481 nmol×h/L, respectively ($p<0.001$). The mean ratio of E_{AUC} for AVONEX™ to Rebif® was 144% with 90% confidence limits of 131 to 159%. The least squares mean E_{max} values for AVONEX™ and Rebif® were 9.5 and 6.9 nmol/L, respectively ($p<0.001$). The mean ratio of E_{max} for AVONEX™ to Rebif® was 138% with 90% confidence limits of 123 to 156%.

All subjects were included in the analysis of safety. Following AVONEX™, 21 of 29 subjects (72%) experienced an adverse event; following Rebif®, 21 of 30 subjects (70%) experienced an event. Adverse events related to the interferon-mediated flu syndrome were seen after treatment with both products. The most commonly reported adverse event was headache: 40% of subjects following Rebif®, 38% of subjects following AVONEX™. Nausea, backache and muscle ache were reported in 24%, 28% and 31%, respectively, of subjects after injection with AVONEX™ and in 10% or fewer of subjects following dosing with Rebif®. The higher

incidence rates of the these latter three symptoms may relate to the significantly higher bioavailability associated with AVONEX™. Injection site reactions were not reported with either treatment.

Together, these results indicate that two proteins that should be similar in their biochemical properties do not possess identical absorption profiles or pharmacodynamic effects following IM administration. Potential explanations for the discrepancy include: (i) unidentified structural differences between the two molecules, (ii) differences in amount of drug administered, or (iii) differences in formulation. With regard to the first possibility, since both molecules are produced by inserting the natural human gene for IFN-β into Chinese hamster ovary cells, it is unlikely that major structural differences exist in the active drug moieties contained in AVONEX™ and Rebif®. Instead, lesser mass amounts of drug in the respective vials could partially explain the lower absorbed dose after Rebif® injection. Based on product labelling, the study subjects received identical doses of each product. However, the specific activity of the IFN-β-1a in Rebif® has been reported to be 3×10^8 units/mg (ie. 300 MU of antiviral activity per milligram of IFN-β-1a protein) whereas AVONEX™ has a specific activity of 2×10^8 units/mg (14). This would indicate that a 6 MU vial of Rebif® contains 20 μg of IFN-β-1a while a 6 MU vial of AVONEX™ contains 30 μg of IFN-β-1a. Direct confirmation of this difference could not be obtained because both products are formulated with greater than a 300-fold excess of HSA which interferes with precise determination of IFN-β-1a concentration.

Yet, this apparent difference in dose mass does not account, in full, for the two-fold greater difference in pharmacokinetic measurements. As discussed below, another known difference that could explain the results is formulation.

Most importantly, the differences in formulation and the observed differences in pharmacokinetic and pharmacodynamic parameters suggest that definite statements regarding the clinical efficacy of Rebif® given IM to patients with multiple sclerosis require clinical studies with Rebif® given by that route of administration. Until such data are available, the findings provided here indicate that simple substitution of IM Rebif® for AVONEX™ at similar labelled doses is not likely to reproduce the therapeutic effects observed with AVONEX™.

B. Single Dose Study

Demographics

Table III summarizes baseline demographics for each group. Age, height and weight were similar across the groups.

Pharmacokinetics

Figure 3 shows mean interferon activity levels by time from the start of the IV infusion. Maximum levels, C_{max}, were detected either at 20 minutes (in one subject) or at 30 minutes, i.e., when the infusion was stopped (in all other subjects). C_{max} ranged from 160 to 640 U/mL. The data were well described by the two-compartment model. From this model, the mean distribution and elimination half-lives were 4 minutes and 4 hours, respectively. The mean volume of distribution was 61.6 L, and mean total clearance was 334 mL/h/kg.

Figure 4 shows mean serum interferon activity levels by time after SC and IM injection. Peak interferon activity levels after IM injection were 40 U/mL in all but one subject, in whom the peak level was 80 U/mL. The mean C_{max} after SC administration was 20 U/mL. However, serum interferon activity levels remained below the limit of detection at all timepoints after injection in 4 of 8 subjects in the SC dosing group. Following IM administration, AUC and C_{max} were significantly greater than after SC administration ($p<0.001$ and $p=0.033$, respectively).

The mean apparent elimination half-life was 10.0 hours after IM injection and 8.6 hours after SC injection, both higher than that seen in the IV infusion group. Since clearance mechanisms are likely to be the same as after IV infusion, these elimination half-life calculations indicate prolonged absorption from the site of IM or SC injection.

An AUC_{IM} greater than AUC_{IV} is an unexpected result, but can be accounted for by the prolonged absorption phase, which can lead to increased serum exposure (see discussion). Bioavailability was not calculated since insufficient data were available to reliably adjust the AUC ratio for the prolonged absorption time. Table II summarizes the pharmacokinetic results.

Pharmacodynamics

Mean serum β_2-microglobulin and neopterin concentrations by time are shown in Figures 5 and 6, respectively. Peak levels of both markers were observed at either 24 or 48 hours

post-dosing. Mean E_{max} for serum β_2-microglobulin was 796 μg/L after IM injection, 628 μg/L after SC injection, and 559 μg/L following IV infusion (p=0.050 for IM *v.* SC; p=0.008 for IM *v.* IV). E_{max} for serum neopterin was 16.0 nmol/L after IM injection, and 12.4 nmol/L after SC injection (p=0.18). E_{max} values are summarized in Table IV.

This single study has defined the single dose pharmacokinetic and pharmacodynamic response profiles of this particular IFN-β-1a product, Avonex™, after IV, SC or IM administration. After a 30 minute IV infusion, IFN-β-1a is rapidly redistributed over a few minutes, then cleared more slowly over several hours. The elimination half-life seen in this study was approximately 4 hours compared to the 1.5 to 5 hours reported previously for other interferon beta products. Similarly, the minimally elevated levels of serum interferon activity after SC administration are consistent with other interferon beta products. However, detectable serum levels of interferon have not been seen after IM administration of these other interferon beta products .[See, e.g., (15)] In the current study, detectable interferon levels were consistently seen after IM injection. In addition, the AUC of serum interferon activity after IM injection was 2 to 3 fold higher than after SC administration.

The finding of an increased absorption after IM compared to SC injection with the IFN-β-1a product used in the current study is not likely to be an intrinsic property of the active drug molecule. In a separate study (7), no difference in the pharmacokinetics or pharmacodynamics were reported between IM and SC injection for Rebif®. The IFN-β-1a molecules used in both the current and this other study are produced by inserting the natural human gene for interferon beta into Chinese hamster ovary cells. As a result, it is unlikely that major structural differences exist in the active drug moieties of the two products. In addition, after IV administration, the pharmacokinetic properties of the Avonex IFN-β-1a used in the current study is similar to those reported previously. Since, the impact of inactive components of the product (i.e. excipients) would be expected to be minimal after IV administration, the similar IV pharmacokinetic parameters for the various interferon beta molecules suggest that the intrinsic pharmacokinetic behavior of these molecules are not substantially different.

The more likely explanation for the ability to deliver interferon to the bloodstream after IM

injection of the current product relates to the inactive product components (i.e., excipients). Paulesu [17] *et. al.* have suggested that the poor absorption of many interferon beta molecules after IM injection may be due to binding of interferon beta to extracellular matrix in the muscle or to inactivation by proteases. They further demonstrated that muscle homogenate can inactivate IFN-β and not IFN-α. This inactivation was partially blocked by albumin, suggesting that manipulation of the formulation in this way could increase the bioavailability of interferon beta after IM injection. The various interferon beta products differ in their formulations and these differences might also affect interaction with local factors in the muscle. For example, both IFN-β-1b and the IFN-β-1a used in the current study are formulated at physiologic pH and contain 15 mg/mL of human albumin as a stabilizer.(18) However, IFN-β-1b also contains dextrose, which IFN-β-1a does not. The second IFN-β-1a product has a pH of 5-5.5 after reconstitution, and contains mannitol and 9 mg/mL of human albumin as stabilizers (15).

Absorption from the SC and IM injection sites were prolonged. As a result, bioavailability for the two routes of administration could not be calculated. The standard method of determining bioavailability is by calculating fraction absorbed (e.g., AUC_{IM}/AUC_{IV}). By this ratio alone, the apparent bioavailability exceeded 100%. However, this approach can lead to an overestimate of bioavailability when the absorption process is substantially slower than the elimination process ("flip-flop" kinetics).(19) Under such circumstances the AUC_{IM} values need to be adjusted by their appropriate rate constant (i.e. the apparent elimination rate constant k_{el} should be substituted with the apparent absorption rate constant k_a). In the current study, such an adjusted bioavailability could not be calculated because of the inter-subject variability in absorption and elimination rates.

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Table I.
Summary of Pharmacokinetic Parameter Analysis

Study Drug	AUC[1] (U×h/mL)	C_{max}[2] (U/mL)	t_{max}[2] (h)
AVONEX™	824	33.8	15
Rebif®	403	15.2	12
Ratio: AVONEX™ to Rebif®	204%	222%	N.A.
90% C.I.	172-243%	172%-285%	N.A.
p-value	< 0.001	<0.001	>0.05

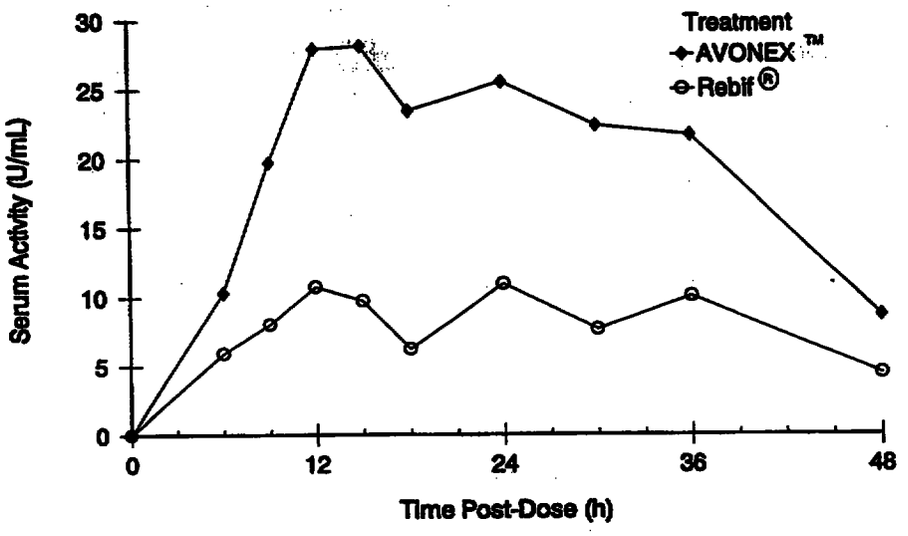
1 Values are least squares means transformed back from the logarithmic scale, based on analysis of variance adjusting for subjects and periods.
2 Values are arithmetic means.

Table II.
Summary of Pharmacodynamic Parameters: Baseline Corrected Serum Neopterin

	E_{AUC}[1] (nmol×h/L)	E_{max}[1] (nmol/L)	t_{max}[2] (h)
AVONEX™	693	9.5	44.9
Rebif®	481	6.9	50.3
Ratio: AVONEX™ to Rebif®	144%	138%	
90% C.I.	131-159%	123-156%	
p-value	<0.001	<0.001	

1 Values are least squares means transformed back from the logarithmic scale, each based on analysis of variance adjusting for subjects and periods. P-values are based on the comparison of the two products estimated from this model.
2 Values are arithmetic means.

Figures 1 and 2.



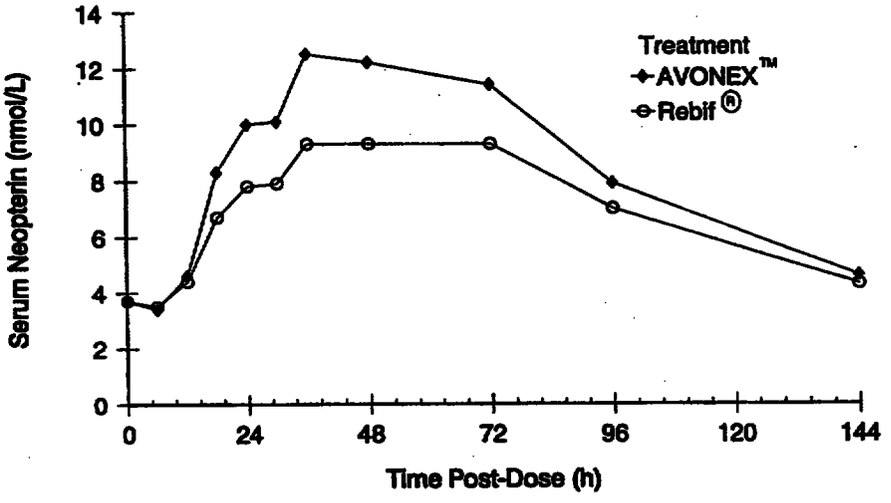


Figure Captions

Fig. 1. Mean Serum activities following IM administration of 6 MU IFN-β-1a in 29 healthy subjects (15 male, 14 female).

Fig. 2. Serum Neopterin Concentrations - Geometric Means versus Time.

Table III. Summary of demographics.

	Route of Administration		
Parameter	Intravenous	Intramuscular	Subcutaneous
Age (years)	27.9 (±6.7)	27.6 (±3.7)	31.5 (±8.4)
Height (cm)	171.5 (±10.3)	168.6 (±8.6)	166.1 (±4.8)
Weight (kg)	68.4 (±10.6)	63.1 (±8.9)	65.9 (±6.9)

Values are mean (± s.d.).

Table IV. Summary of pharmacokinetic and pharmacodynamic parameters.

	Route of Administration		
Parameter	Intravenous	Intramuscular	Subcutaneous
C_{max} (U/mL)	262 (±45)	44 (±4)	20 (±7)
AUC (h·U/mL)	537 (±40)	1352 (±111)	478 (±119)
Elimination $T_{½}$ (h)	4.0 (±1.6)	10.0 (±0.8)	8.6 (±1.0)
Distribution $T_{½}$ (h)	0.07 (±0.02)	-	-
Clearance (mL/h/kg)	334 (±18)	-	-
Vd (L)	61.6 (±16.7)	-	-
β_2-microglobulin: E_{max} (µg/L)	559 (±70)	796 (±58)	628 (±39)
Neopterin: E_{max} (nmol/L)	-	16.0 (±2.0)	12.4 (±1.5)

Values are mean (± s.e.m.).

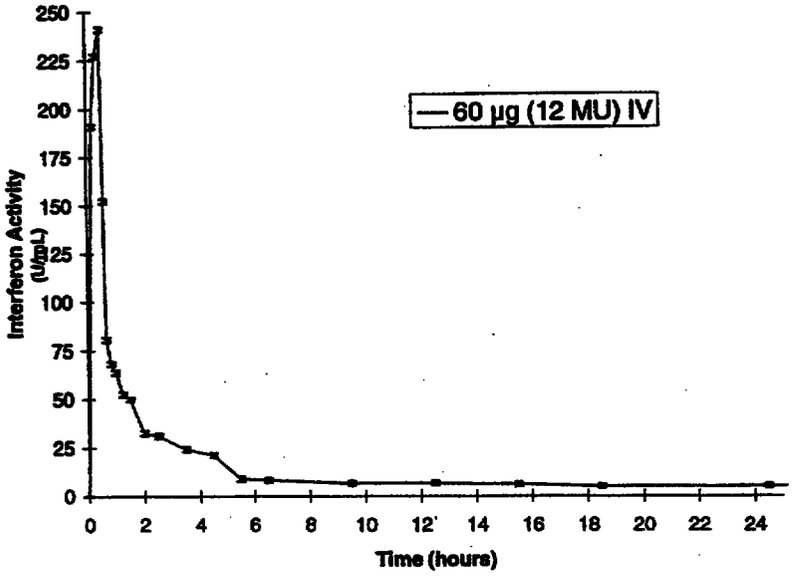


Figure 3.

Mean (± s.e.m.) serum interferon activity levels by time from the start of 30 minute IV infusion of 60 µg (12 MU) of IFN-β-1a (n=8 subjects).

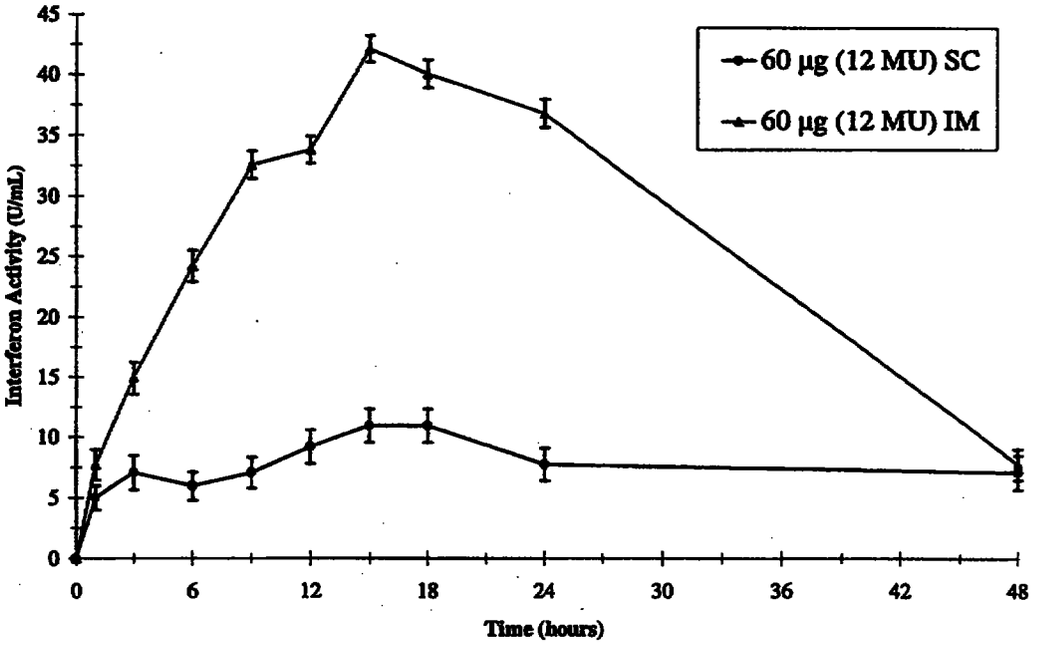


Figure 4.

Mean (± s.e.m.) serum interferon activity levels following IM or SC injection of 60 µg (12 MU) of IFN-β-1a (n=8 subjects per group).

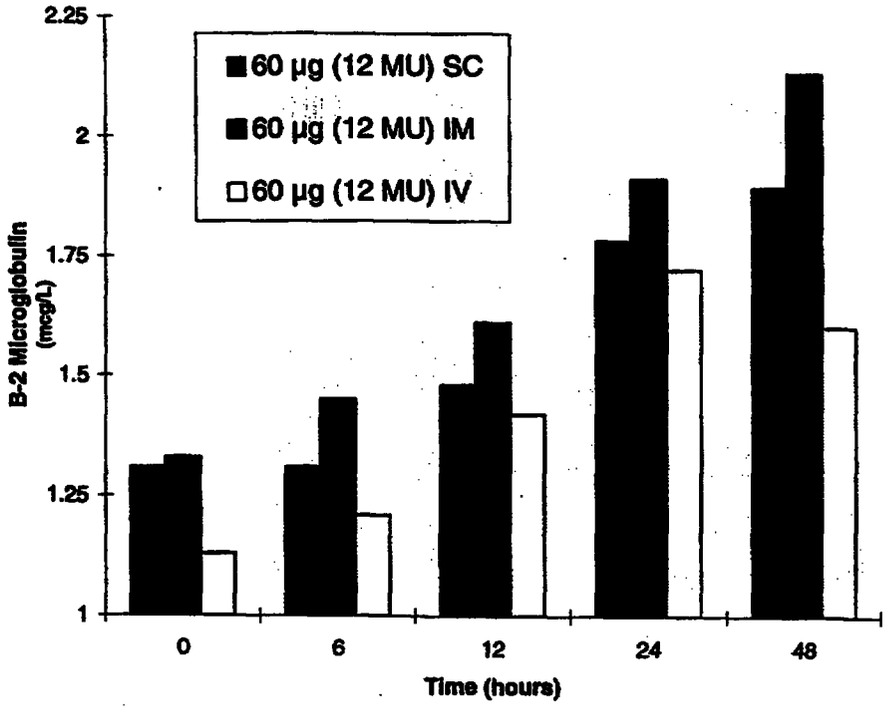


Figure 5.

Mean serum β_2-microglobulin concentrations by time following single administrations of 60 µg (12 MU) of IFN-β-1a (n=8 subjects per group).

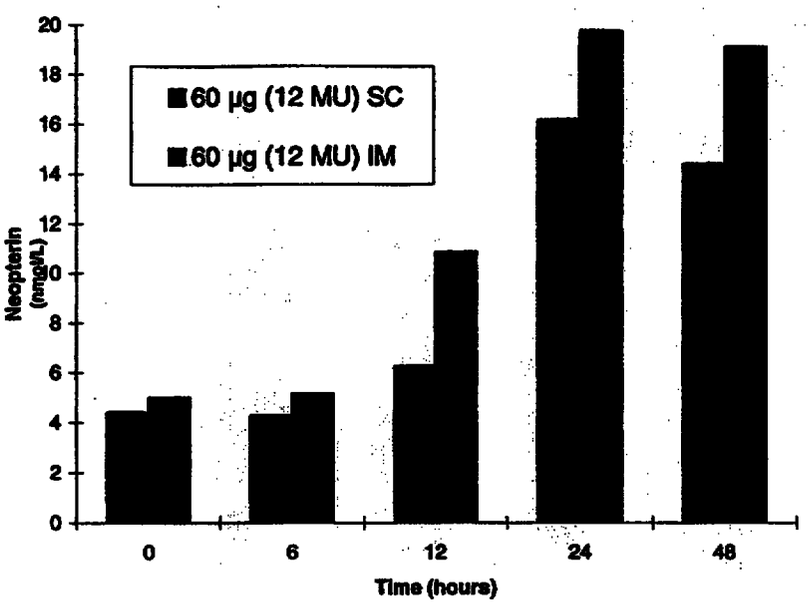


Figure 6.

Mean serum neopterin concentrations by time following single administrations of 60 µg (12 MU) of IFN-β-1a (n=8 subjects per group).

CLAIMS

1. A liquid composition comprising a buffer of pH about 7.2, recombinant interferon-beta and 15 mg/ml of human serum albumin, wherein the composition is characterized as having an enhanced pharmackinetic profile as compared to pharmacokinetic profile of a liquid composition of interferon-beta of pH about 5.0 containing 9 mg/ml human serum albumin, said enhanced pharmacokinetic profile defined in Table I, and further wherein the composition is characterized as having enhanced pharmacodyamic profile of a liquid composition of interferon-beta of pH about 5.0 containing 9 mg/ml human serum, said enhanced pharmacodyamic profile defined in Table II.

2. The composition of claim 1, wherein the composition is contained within a vessel.

3. The composition of claim 2, wherein the vessel is a syringe.

4. A liquid pharmaceutical composition contained within a storage vessel, the liquid suitable foe parental administration to mammals and consisting essentially of an effective amount of interferon-beta, a buffer maintaining the pH at about 7.2, and human serum albumin, wherein the interferon-beta has been subjected to prior lyophilization and wherein the composition is characterized as having enhanced pharmacokinetic profile as compared to pharmackinetic profile of a liquid composition of interferon-beta of pH 5.0 containing 9 mg/ml human serum albumin, said enhanced pahrmackinetic profile defined in Table I, and further wherein the composition is characterized as having enhanced pharmacodyamic profile as compared to a pharmacodynamic profile of a liquid composition of interferon-beta of about 5.0 containing 9 mg/ml human serum albumin, said enhanced pharmacodynamic profile defined in Table II.

5. The liquid composition of claim 4, wherein the storage vessel is a syringe.

* C L M S *

6. The liquid composition of claim 4, which is sterile.

7. The liquid composition of claim 4, wherein the interferon-beta is human recombinant interferon-beta.

8. A kit for parental administration of a liquid interferon formulation, wherein the kit comprises: a) a vessel containing the liquid formulation of claim 4 and b) instructions for use thereof.

9. The kit of claim 8, further comprising: c) an alcohol swab; d) a needle; and at least one adhesive bandage.

