Comparative Pharmacokinetics and Pharmacodynamics of Two Recombinant Human Interferon Beta-1a (IFNβ-1a) Products Administered Intramuscularly in Healthy Male and Female Volunteers

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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* (IFNβ-1b, containing a genetically engineered serine substitution for cystine at position 17), and recombinant human IFN-β produced in Chinese hamster ovary cells (IFNβ-1a, containing the natural human amino acid sequence). Both n-IFN-β and IFNβ-1a are glycosylated with a single N-linked complex carbohydrate moiety whereas IFN-β-1b is not glycosylated.

AVONEX™, one IFNβ-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 IFNβ-1a product, Rebif®, is being given subcutaneously (SC) in ongoing phase 3 trials in the treatment of multiple sclerosis. Previous studies with AVONEX™ and Rebif® had raised concerns that while these products are both designated as IFNβ-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 that AVONEX™ and Rebif® are not equivalent when administered intramuscularly.

METHODS

Study Design

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

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United Kingdom and adhered to the tenets of the Declaration of Helsinki.

Study Drug

AVONEX™ was supplied as a sterile lyophilized powder containing IFNβ-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 IFNβ-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.

Assay Methods

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 in 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 infection by 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.

Pharmacokinetic and Statistical Methods

Standard descriptive analysis of serum interferon activity data for both IFNβ-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.

RESULTS AND DISCUSSION

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 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% ($p < 0.001$).

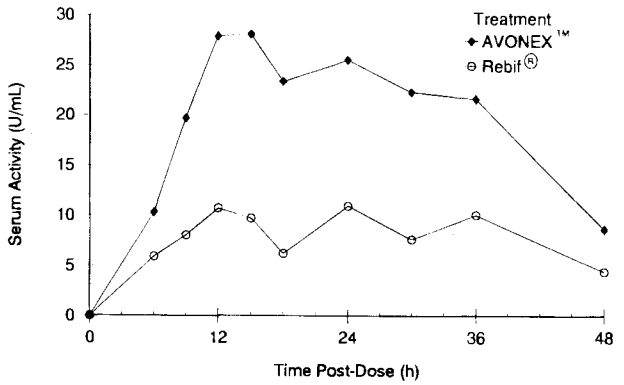

Fig. 1. Mean serum activities following IM administration of 6 MU IFNβ-1a in 29 healthy subjects (15 male, 14 female).

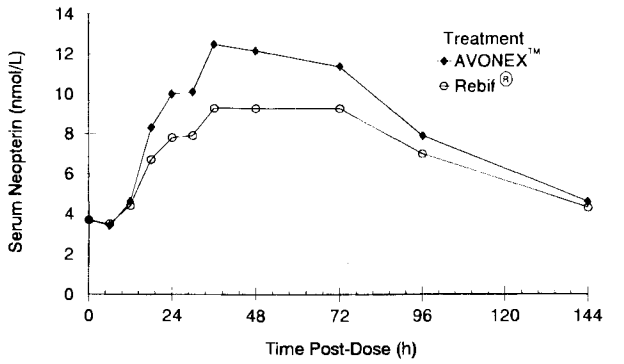

Fig. 2. Serum neopterin concentrations—geometric means versus time.

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 likely relate to the significantly higher bioavailability associated with AVONEX™. Injection site reactions were not reported with either treatment.

Together, these results indicate that two IFNβ-1a 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

Table I. Summary of Pharmacokinetic Parameters

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

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

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

	E_{AUC}[a] (nmol·h/Ml)	E_{max}[a] (nmol/L)	t_{max}[b] (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	

[a] 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.
[b] Values are arithmetic means.

Rebif® injection. Based on product labelling, the study subjects received identical doses of each product. However, the specific activity of the IFNβ-1a in Rebif® has been reported to be 3×10^8 units/mg (ie. 300 MU of antiviral activity per milligram of IFNβ-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 IFNβ-1a while a 6 MU vial of AVONEX™ contains 30 μg of IFNβ-1a. Direct confirmation of this difference could not be obtained because both products are formulated with greater than a 400-fold excess of HSA which interferes with precise determination of IFNβ-1a concentration.

Yet, this apparent difference in dose mass does not account, in full, for the two-fold greater difference in pharmacokinetic measurements. Another known difference that could explain the results is formulation. 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 could contribute to altered absorption of IFN-β after IM injection by affecting binding to the muscle extracellular matrix and/or inactivation by pH-dependent proteases (17).

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™.

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