In Vivo Pharmacology and Toxicology Evaluation of Polyethylene Glycol-Conjugated Interferon β-1a

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ABSTRACT

Human interferon (IFN) β has well established beneficial effects in treating relapsing forms of multiple sclerosis, but current first-line treatment requires frequent (from daily to weekly) parenteral administration. A 20-kDa polyethylene glycol (PEG)-conjugated IFN β-1a (PEG-IFN β-1a) is being developed to decrease the frequency of administration and improve patient convenience and compliance. We present pharmacokinetic (PK) and pharmacodynamic (PD) parameters, immunogenicity, convenience and compliance. We present pharmacokinetic and pharmacodynamic (PD) parameters, immunogenicity, and safety of PEG-IFN β-1a in Rhesus monkeys in support of a phase 1 clinical trial. Two single-dose PK/PD studies and one 5-week repeat-dose toxicity study compliant with good laboratory practice were conducted. The PK of IFN β-1a and PEG-IFN β-1a were modeled with a two-compartment model, and the link between drug concentration and neopterin response (PD marker) was described with an indirect stimulatory model.

Introduction

Human interferon (IFN) β has well established beneficial effects when used to treat patients with relapsing forms of multiple sclerosis (MS), including reducing the development of brain magnetic resonance imaging lesions (Jacobs et al., 1996), reducing clinical relapse rates (Jacobs et al., 1996; PRISMS Study Group, 1998), and slowing the advance of physical disability (Jacobs et al., 1996; PRISMS Study Group, 1998). The mechanism of action in MS is not fully understood despite extensive research, because of the heterogeneous nature of the disease and the pleiotropic activities of the protein. The antiproliferative and immunomodulatory activities of IFN β may be relevant to the therapeutic effect (Axtell and Steinman, 2008). For some patients, injection-related concerns and treatment-associated side effects, such as flu-like symptoms, remain as barriers to compliance and effective treatment (Coyle, 2008). In addition, all currently approved parenteral treatments for MS require frequent (from daily to weekly) administration, which can further inconvenience patients already suffering from a debilitating chronic disease. Thus, there remains a significant unmet need for effective, safe, well tolerated, and convenient therapies for patients with MS (Mohr et al., 2001; Lugaresi, 2009).

Polyethylene glycol (PEG) conjugation (PEGylation) is a method to increase the apparent size of a biomolecule and PEG-IFN β-1a showed greater exposure, longer half-life, lower clearance, and reduced volume of distribution than unmodified IFN β-1a. Consistent with the pharmacology of type I IFNs, PEG-IFN β-1a resulted in the elevation of neopterin concentration, a transient body temperature increase, and a reversible lymphocyte count decrease. As expected, neutralizing antibodies to PEG-IFN β-1a formed in almost all monkeys after 5 weeks of treatment, which resulted in significantly reduced drug exposure and abrogation of neopterin induction. There were no drug-related adverse effects at doses up to 100 μg/kg (11 MIU/kg) given subcutaneously or intramuscularly once weekly for 5 weeks. The no-observed-adverse-effect level was determined to be 100 μg/kg (11 MIU/kg), the highest dose tested.
reduce its glomerular filtration rate, thereby extending the biomolecule’s half-life, enhancing its in vivo efficacy through prolonged systemic drug exposure, and enabling less frequent dosing (Bailon and Won, 2009). PEG-conjugated (PEGylated) IFN-β-1a (PEG-IFN-β-1a) was developed by attaching 20-kDa methoxy-PEG-O-2-methylpropionaldehyde to the α-amino group of the N terminus of IFN-β-1a (Baker et al., 2006), with the aim of providing a less frequent dosing regimen and improved convenience for patients with MS. The N terminus of IFN-β-1a is not critical for binding to the type 1 IFN receptor (Runkel et al., 2000). In vitro evaluation demonstrated that PEG-IFN-β-1a retained significant in vitro activity (approximately 50%) in antiviral and antiproliferative assays compared with the unmodified protein, whereas PEG-IFN-β-1a in vivo efficacy was enhanced compared with IFN-β-1a in a mouse tumor angiogenesis model (Baker et al., 2006).

Rhesus monkeys have been shown to elicit a robust pharmacologic response to IFN-β-1a and have been used for pharmacokinetic (PK), pharmacodynamic (PD), and safety evaluation of IFN-β-1a (http://www.accessdata.fda.gov/drugsatfda_docs/label/2007/103628s5115lbl.pdf). Rhesus safety evaluation of IFN-β-1a and IFN-β-1a has been shown to increase after administration of IFN-β-1a (Martin et al., 2002; Biogen Idec, data on file). Neopterin was selected as the pharmacodynamic marker for the studies described here because it shows lower interanimal variability in response based on historic data for IFN-β-1a.

The relationship between IFN-β-1a concentrations and subsequent neopterin response has been characterized with an integrated PK/PD model (Mager and Jusko, 2002; Mager et al., 2003), which was built on simplified biological processes. However, the model included many hypothetical intermediate compartments and was overparameterized, and final parameter estimates relied on several fixed values derived from the literature. A minimal model was later proposed for a 40-kDa PEG-IFN-β-1a conjugate administered to cynomolgus monkeys, in which the receptor-mediated drug disposition was simplified to a linear two-compartment PK model with the intermediate compartments between serum drug concentration and stimulatory effect and an intermediate compartment for a neopterin precursor removed (Mager et al., 2005). This simplified PK/PD model was adopted with slight modifications for the current study, including the addition of transit compartments to describe neopterin lag time (Savic et al., 2007) and incorporation of inhibitory feedback of neopterin on the stimulatory effect by IFN-β-1a or PEG-IFN-β-1a (Mager et al., 2003).

Herein we report the results of nonclinical studies aimed at evaluating the PK, PD, immunogenicity, and safety of PEG-IFN-β-1a in Rhesus monkeys. The results provided preclinical support for two phase 1 clinical trials of PEG-IFN-β-1a.

**Materials and Methods**

**PEG-IFN β-1a and IFN β-1a.** Both PEG-IFN β-1a and IFN β-1a were manufactured by Biogen Idec (Cambridge, MA) and formulated in 20 mM acetic acid/sodium acetate, pH 4.8, 150 mM arginine-HCl, 0.005% (w/v) Polysorbate 20. Laboratory-scale PEGylation, purification, and characterization of PEG-IFN-β-1a has been described elsewhere (Baker et al., 2006). The amino acid sequence of IFN-β-1a is available in the DrugBank database (Knox et al., 2011).

**In Vitro Potencies of PEG-IFN β-1a and IFN β-1a.** The in vitro-specific antiviral activity of PEG-IFN β-1a and IFN β-1a was determined using a cytopathic effect assay that measures the ability of the protein to protect human lung carcinoma A549 cells challenged with encephalomyocarditis virus. In the assay, A549 cells were added to wells of a 96-well microtiter plate and incubated for 15 to 20 h. PEG-IFN β-1a or IFN β-1a standards, controls, and serum samples, diluted in Dulbeco's modified Eagle's medium supplemented with fetal bovine serum, were added to each well. After an additional incubation, encephalomyocarditis virus was added to the assay plates and incubated for 30 h. After the viral incubation, the plates were stained with 0.75% crystal violet in formaldehyde fixative. The plates were examined visually to determine the lowest concentration of PEG-IFN β-1a or IFN β-1a protecting the cells against virus cytopathicity. The last positive well of the standards was used as the standard detection limit of the assay. To determine potency of PEG-IFN β-1a or IFN β-1a in the samples, the sample dilution in the last positive well was multiplied by the standard detection limit and the sample dilution factor. Specific activity for the samples was determined by dividing the sample potency by the sample protein concentration.

**Rhesus Monkey PK, PD, Immunogenicity, and Toxicology Studies.** Three studies using Rhesus monkeys were conducted to evaluate PK, PD, immunogenicity, and toxicity of PEG-IFN-β-1a. All aspects of the animal studies were fully compliant with the U.S. Drug Administration Animal Welfare Act and the conditions specified in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996).

In study 1 (PK/PD), four groups of Rhesus monkeys (five males per group) received a single dose of PEG-IFN-β-1a at 10.8 μg/kg (1 MIU/kg) or IFN-β-1a at 5 μg/kg (1 MIU/kg) via subcutaneous or intramuscular injection. For assessment of serum drug and neopterin concentrations, blood samples were obtained before dosing and at 1, 2, 4, 8, 24, 32, 48, 72, 120, and 168 h postdose. In study 2 (PK/PD/immunogenicity), four groups of Rhesus monkeys (two males and two females per group) received a single-dose PEG-IFN-β-1a treatment at 2, 10, and 100 μg/kg (equivalent to 0.22, 1.1, and 11 MIU/kg, respectively) via subcutaneous injection and at 100 μg/kg via intramuscular injection. The blood sampling schedule was the same as in study 1 for assessment of serum drug and neopterin concentrations. In addition, serum samples were collected before dosing and at 14 and 28 days postdosing for assessment of anti-PEG-IFN-β-1a binding antibodies (BAbs) and neutralizing antibodies (NAbs). Study 3 (PK/PD/immunogenicity/toxicology) was a 5-week repeat-dose toxicity study conducted according to good laboratory practice. This study incorporated subcutaneous (all doses) and intramuscular (control and high dose only) routes of administration, because both routes were to be evaluated in the initial phase 1 clinical study to determine the safety, tolerability, PK, and PD of PEG-IFN-β-1a in humans. Five groups of monkeys received five weekly doses of PEG-IFN-β-1a. Group 1 received intramuscular and subcutaneous administration of vehicle control; groups 2 to 4 received subcutaneous administration of PEG-IFN-β-1a at 2, 10, and 100 μg/kg (equivalent to 0.22, 1.1, and 11 MIU/kg); and group 5 received intramuscular administration of PEG-IFN-β-1a at 100 μg/kg. There were four male and four female monkeys per group for the main study and an additional two males and two females per group for recovery for groups 1, 4, and 5.

For assessment of PEG-IFN-β-1a concentration, serum samples were obtained before dosing and at 4, 8, 24, 120, and...
168 h postdose for the first dose (dosing day defined as day 1). For the last dose (day 29), the same schedule was used except that groups 1 through 4 skipped the 4-h postdose sample, and group 5 skipped the 8-h postdose sample. In addition, for the third (day 15) and fourth (day 22) doses, predose (groups 1–5), 4-h (groups 1–4), and 8-h (group 5) samples were taken to assess drug exposure. Neopterin concentration was measured from serum samples before dosing, at 24 h postdosing for the first, third, and fifth doses, at 168 h postdosing for the fifth dose, and at the end of the study (day 65) from the recovery animals only. For assessment of anti-PEG-IFN β-1a BAbs and NAbS, blood samples were collected before day 1 dosing, on days 15 and 29 at predose, on day 35, and on day 65 from recovery animals.

The main study’s necropsy (4/sex/group) was on day 36, and the recovery necropsy was on day 65 for groups 1, 4, and 5 (2/sex/group). In addition to PK, PD, and immunogenicity determination, study endpoints included twice-daily cage-side observation, daily food consumption, physical and ophthalmic exams (pretstudy and once before each necropsy), and body weight and ECGs (twice pretstudy and weekly thereafter). Blood samples for evaluation of serum chemistry, hematology, and coagulation parameters were collected from all animals twice prestudy (days −10 and −2), on day 1 (at 8 h postdose for all groups), on days 15, 22, and 29 (at 8 h postdose for groups 1–4 and at 4 h postdose for group 5), and before each necropsy. Body temperature was taken twice prestudy (days −28 and −25), before dosing, and at 4 and 8 h postdosing on days 1, 8, 15, 22, and 29. Blood samples were also taken for neopterin measurement before dosing, at 24 h postdosing for the first, third, and fifth doses, 168 h postdosing for the fifth dose, and at the end of the study (day 65). Urine samples were collected for analysis (bladder puncture) before each necropsy.

At each necropsy on days 36 and 65, a full gross evaluation was conducted. Organs were collected and weighed, and a comprehensive panel of tissue samples was taken from all animals, preserved in neutral-buffered 10% formalin (except for the eyes, which were preserved in Davidson’s fixative for optimum fixation), embedded in paraffin, stained with hematoxylin and eosin, and examined by a board-certified veterinary pathologist.

**Determination of Serum Concentration of PEG-IFN β-1a and IFN β-1a.** The PK assay for PEG-IFN β-1a and IFN β-1a was a one-step sandwich enzyme-linked immunosorbent assay (ELISA) that uses selected kit components from a commercially available IFN β Direct ELISA kit (Biosource, manufactured by Fujirebio Diagnostics, Malvern, PA and distributed by Invitrogen, Carlsbad, CA). Affinity-purified goat anti-human IFN β was precoated on a polystyrene 96-well plate by the manufacturer. Assay standards and quality controls prepared from PEG-IFN β-1a and IFN β-1a and study samples were added to the plate. PEG-IFN β-1a or IFN β-1a, captured from the serum, was detected using a mouse anti-human IFN β monoclonal antibody conjugated to horseradish peroxidase (HRP). Quantitation of quality controls and study samples was carried out by interpolation to the standard curve using a validated Softmax Pro 4.3.1 data reduction software program. The assay was validated for use with Rhesus monkey serum samples. The quantitation range was 0.521 to 100 ng/ml, and the precision of the assay (%CV of assay controls) was within 20%.

**Immunogenicity Assay.** Detection of anti-IFN β-1a antibodies was carried out using a screening ELISA to detect BAbs and a reporter gene-based assay to detect NAbS. To detect BAbs, PEG-IFN β-1a was captured by a murine anti-human IFN β antibody coated on a 96-well microtiter plate. The plates were then incubated with assay quality controls and study samples. Antibodies to IFN β-1a were detected using a polyclonal anti-human IgG (cross-reacting with monkey IgG) conjugated to HRP. NAbS were characterized by their ability to inhibit the binding of IFN β-1a to the type I interferon receptor using a quantitative Neutekbio iLite øβ human type I interferon activity detection kit (Biomonitor Ltd., Galway, Ireland). The binding of IFN β-1a to its receptor, expressed on stably transfected human cells, initiates an intracellular cascade of signal transduction events that induces the luciferase gene under the transcriptional control of an interferon-sensitive response element, resulting in the production of the luciferase enzyme in a dose-dependent manner. NAbS, present in the serum, inhibit the binding of IFN β-1a to its receptor, thereby inhibiting the production of luciferase. The NAb assay was carried out in two steps, a screening assay followed by a titration assay. Samples with a percentage inhibition at or above the assay cut point were considered positive and were further evaluated in a titration assay by serial dilution to determine titer values.

**Calculation of PK and PD Parameters Using Noncompartmental Analysis.** Noncompartmental PK analyses were carried out using WinNonlin Professional Versions 4.0.1 and 5.0 software (Pharsight, Mountain View, CA). Data were analyzed using noncompartmental analysis (NCA) with an extravascular input model (WinNonlin model 200). The parameters included maximum serum concentration (Cmax), the time to reach Cmax (Tmax), area under the concentration time curve from time 0 to infinity (AUCinf), clearance (CL/F), and elimination half-life (t1/2). For nefopterin, the parameters included postdose peak concentration (Npeak), time to reach Npeak (Nt_max), and area under the neopterin concentration time curve from time 0 to 168 h postdose (Nt_AUC_168h).

**PK Model.** The PK/PD model was built sequentially, and the integrated PK/PD model is shown in Fig. 1. The PK model consists of a two-compartment model with linear absorption rate (Ka), elimination rate (Ke), and intercompartment rates, K12 and K21. The drug concentration is described by the following differential equations:

$$\frac{dA_D}{dt} = -Ka \cdot A_D \cdot A_{DOSE}$$

$$\frac{dA_C}{dt} = Ka \cdot A_D \cdot A_{DOSE} + K_{21} \cdot A_{P} - K_{12} \cdot A_C - Ke \cdot A_C$$

$$\frac{dA_P}{dt} = K_{12} \cdot A_C - K_{21} \cdot A_P$$

**Reparameterization:**

$$A_C = C_P \cdot V_C$$

$$K_{12} = Q/V_C$$

$$K_{21} = Q/V_P$$

$$Ke = CL/V_C$$

**Initial values:**

$$A_{DOSE}(0) = \text{administered dose}$$

$$A_C(0) = 0$$
Fig. 1. Integrated PK/PD model after a single subcutaneous or intramuscular administration in Rhesus monkeys. The PK component consists of a linear two-compartment model. The PD model is a stimulatory indirect response model, where the initial delay of neopterin response is modeled with five transit compartments. CL, clearance; EC50, drug concentration to achieve 50% of the maximum stimulatory effect; Emax, maximum stimulatory effect by IFN β-1a or PEG-IFN β-1a; IC50, neopterin concentration to inhibit 50% of the stimulatory effect from IFN β-1a or PEG-IFN β-1a; Ka, absorption rate; Ke, elimination rate; KTR, first-order rate exiting transit compartment; LOSS, first-order loss rate; NEOP, neopterin concentration; Q represents the central compartment volume of distribution, and CL represents the total clearance. The bioavailability of the intramuscular route, F (intramuscular), was fixed as 1. The F (subcutaneous), Ka (intramuscular), Ka (subcutaneous), CL, Vc, Vp, and Q were to be estimated. Intercompartment variance (σ²) for the PK parameters was assumed to be log-normal distributed and added one at a time, judged by numerical stability and decrease of objective function value. The final IFN β-1a PK model included σ² for Vc and Q, with a covariance between the two; the final PEG-IFN β-1a PK model included σ² for CL and Q, with a covariance between the two. The residual error was modeled using an additive error model on the log-transformed PK data.

**PD Model.** The pharmacodynamic component of the model is an indirect stimulatory response model (Dayneka et al., 1993; Mager et al., 2003, 2005) driven by the fixed individual empirical Bayes PK parameters as input in the dataset. The initial lag time for neopterin response was modeled by five transit compartments (Savic et al., 2007). The neopterin concentration was described by the following equations:

$$A_p(0) = 0$$  \hspace{1cm} (10)

in which \(A_p\) represents the amount of drug at the injection site, \(F\) represents the relative bioavailability, \(A_p\) represents the amount of drug in the central compartment, \(Q_{P}\) represents the serum drug concentration, \(Q\) represents the intercompartment clearance, \(V_{C}\) represents the central compartment volume of distribution, \(V_{P}\) represents the peripheral compartment volume of distribution, and \(CL\) represents the total clearance. The bioavailability of the intramuscular route, \(F\) (intramuscular), was fixed as 1. The \(F\) (subcutaneous), \(Ka\) (intramuscular), \(Ka\) (subcutaneous), \(CL\), \(V_{c}\), \(V_{p}\), and \(Q\) were to be estimated. Intersubject variance (\(\sigma^2\)) for the PK parameters was assumed to be log-normal distributed and added one at a time, judged by numerical stability and decrease of objective function value. The final IFN β-1a PK model included \(\sigma^2\) for \(V_{c}\) and \(Q\), with a covariance between the two; the final PEG-IFN β-1a PK model included \(\sigma^2\) for \(CL\) and \(Q\), with a covariance between the two. The residual error was modeled using an additive error model on the log-transformed PK data.

$$\text{Effect} = \frac{E_{\text{max}} \cdot Cp}{EC_{50} + Cp} \cdot \left(1 - \frac{\text{NEOP}}{IC_{50} + \text{NEOP}}\right)$$  \hspace{1cm} (16)

for time > Tau

Reparameterization:

$$\text{SYN} = \text{BSL} \cdot \text{loss}$$  \hspace{1cm} (17)

$$KTR = 5/\text{MTT}$$  \hspace{1cm} (18)

Assuming stationary initial conditions:

$$\frac{dTR1}{dt} = \frac{dTR2}{dt} = \ldots = \frac{dTR5}{dt} = \frac{d\text{NEOP}}{dt} = 0$$  \hspace{1cm} (19)

Initial values:

$$\text{TR1}(0) = \text{TR2}(0) = \ldots = \text{TR5}(0) = \frac{\text{SYN}}{KTR}$$  \hspace{1cm} (20)

$$\text{NEOP}(0) = \text{BSL}$$  \hspace{1cm} (21)

where \(TR1\) through \(TR5\) represent the neopterin concentration in the respective transit compartment, \(SYN\) is the baseline zero-order neopterin synthesis rate, \(effect\) is the stimulation effect by IFN β-1a or PEG-IFN β-1a, \(NEOP\) is the serum neopterin concentration, \(loss\) represents the first-order elimination rate of serum neopterin, \(E_{\text{max}}\) is the maximum stimulation effect, \(EC_{50}\) is the drug concentration to reach 50% of the maximum stimulation effect, \(KTR\) is the first-order rate constant exiting each transit compartment, \(BSL\) is the neopterin baseline concentration before dosing, \(MTT\) is the mean time delay in the transit compartment, \(IC_{50}\) is the neopterin concentration that inhibits the stimulation effect by 50%, and \(Tau\) is the time delay for the neopterin inhibitory feedback to occur. Initial modeling without neopterin inhibitory feedback resulted in overestimating the neopterin concentration – 2 to 3 days after PEG-IFN β-1a dosing. It is known that continuous exposure of interferon reduces the magnitude of neopterin response in both humans and monkeys (Rothuizen et al., 1999; Mager et al., 2003). This phenomenon is attributed to either the down-regulation of the type 1 IFN receptor (Pestka et al., 1987) or inhibitory feedback by prolonged elevation of neopterin concentrations (Liberati et al., 1988). Modeling of the inhibitory effect was attempted by either linking to the drug concentration or the neopterin concentration, with the latter approach providing more
robust parameter estimates, i.e., less sensitive to the initial estimate. BSL, loss, $E_{\text{MAX}}$, $E_{50}$ (IFN $\beta$-1a), $IC_{50}$, MTT (IFN $\beta$-1a), and MTT (PEG-IFN $\beta$-1a) were to be estimated. Initial efforts to estimate $\tau$ in the model resulted in zero gradients. To optimize $\tau$, it was fixed at different values in the range of 2 to 48 h with a 2 h increment and the model predictions were compared. The $\tau$ was fixed as 22 h in the final model, which showed one of the best predictions and was similar to the estimated delay time (29.3 h) in the literature (Mager et al., 2003). Inclusion of $\omega^2$ followed the same procedure as in the PK analysis, and the final model consisted of $\omega^2$ for BSL and $E_{\text{MAX}}$ and a covariance between the two, assuming log-normal distribution. The residual error was modeled using an additive error on the log-transformed neopterin concentration data.

Data Analysis. All PK/PD analyses were carried out using a nonlinear mixed effects modeling program (NONMEM, version VII, level 1.2; ICON Development Solutions, Ellicott City, MD) (Beal et al., 1989–2006; Bauer, 2009) on an Intel-based computer with Intel Visual Fortran Compiler Professional software 11.1.0.048 (Intel, Santa Clara, CA). A first-order conditional estimation method was used for parameter estimation.

Visual Predictive Check. To generate a visual predictive check (VPC) plot for model diagnostics, 1000 simulations were carried out in NONMEM with the final PK and PD models. The observed data and simulated data were processed in R (version 2.11.1; R Foundation for Statistical Computing, Vienna, Austria). PK VPC plots were stratified by drug and route and normalized by dose; PD VPC plots were stratified by drug, route, and dose.

Comparative PK/PD of PEG-IFN $\beta$-1a and IFN $\beta$-1a in Rhesus Monkeys. In study 1, PEG-IFN $\beta$-1a was compared with IFN $\beta$-1a, after a single-dose injection at 1 MU/kg via the intramuscular or subcutaneous routes. The mean serum drug concentration-time curves are shown in Fig. 2A. PEG-IFN $\beta$-1a peaked ~4 h postdose with intramuscular administration and ~15 h postdose with subcutaneous administration, sustained approximate peak concentrations for approximately 0.5 to 1 day, and declined with a $t_{1/2}$ of approximately 20 h. In contrast, IFN $\beta$-1a was absorbed more rapidly than PEG-IFN $\beta$-1a, peaking within 2 h postdose (intramuscularly and subcutaneously). The concentration of IFN $\beta$-1a declined rapidly with a $t_{1/2}$ of ~7 h after the peak, with no apparent plateau phase. Based on the NCA parameters, both AUC$_{\text{inf}}$ and $C_{\text{max}}$ increased significantly after incorporation of a 20-kDa PEG at the N terminus of IFN $\beta$-1a, compared with the unmodified protein (Table 1). For the intramuscular and subcutaneous routes of administration, the mean AUC$_{\text{inf}}$ (mass normalized) of PEG-IFN $\beta$-1a was approximately 36- to 43-fold greater, respectively, and the mean $C_{\text{max}}$ (mass normalized) was approximately 6- to 7-fold greater, respectively. PEGylation reduced CL/F by 35- and 41-fold for the intramuscular and subcutaneous routes, respectively.

The VPC of PK models is presented in Fig. 3, and the parameter estimates are listed in Table 2. Both IFN $\beta$-1a and PEG-IFN $\beta$-1a were well described using a linear two-compartment model, with well aligned median values between the observed and simulated drug concentrations (Fig. 3). There was a slight overestimation of the intersubject and/or intrasubject variability for the IFN $\beta$-1a subcutaneous group, resulting in a wider 5th to 95th percentile range than the observed data at the terminal phase; however, given the small sample size ($n = 5$), the model estimates were considered acceptable. As expected, the Ka of IFN $\beta$-1a was larger than that of PEG-IFN $\beta$-1a, especially for the subcutaneous route, where a ~15-fold decrease was observed, equivalent to a 15-fold increase in the absorption half-life. The $V_{C}$, $V_{P}$, and

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**TABLE 1**

Mean pharmacokinetic parameters from noncompartmental analysis with S.D. shown in parentheses.

The PK parameters of PEG-IFN $\beta$-1a were consistent across studies (referring to the first dose only for the repeat-dose study), therefore, pooled parameters are presented.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Route</th>
<th>n</th>
<th>AUC$_{\text{inf}}$/Dose (ng/ml/h)</th>
<th>$C_{\text{max}}$/Dose (ng/ml)</th>
<th>CL/F (ml/h/kg)</th>
<th>$t_{1/2}$ (h)</th>
<th>$t_{\text{max}}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN $\beta$-1a</td>
<td>IM</td>
<td>5</td>
<td>3.53 (1.23)</td>
<td>0.829 (0.619)</td>
<td>308 (95)</td>
<td>6.30 (2.95)</td>
<td>1.60 (0.55)</td>
</tr>
<tr>
<td>IFN $\beta$-1a</td>
<td>SC</td>
<td>5</td>
<td>2.53 (0.44)</td>
<td>0.347 (0.147)</td>
<td>405 (69.9)</td>
<td>7.42 (1.68)</td>
<td>1.60 (0.55)</td>
</tr>
<tr>
<td>PEG-IFN $\beta$-1a</td>
<td>IM</td>
<td>21</td>
<td>126 (35.8)</td>
<td>4.79 (1.64)</td>
<td>8.91 (3.95)</td>
<td>20.7 (5.30)</td>
<td>4.29 (2.10)</td>
</tr>
<tr>
<td>PEG-IFN $\beta$-1a</td>
<td>SC</td>
<td>45</td>
<td>108 (31.7)</td>
<td>2.48 (1.65)</td>
<td>10.0 (2.97)</td>
<td>19.1 (4.67)</td>
<td>14.6 (10.0)</td>
</tr>
</tbody>
</table>

IM, intramuscular; SC, subcutaneous.
CL were decreased by 15-, 11-, and 37-fold, respectively, after PEGylation.

The mean serum concentration-time curves of neopterin after a single injection of PEG-IFN β-1a or IFN β-1a via the intramuscular or subcutaneous route at 1 MIU/kg are shown in Fig. 2B. Single-dose intramuscular or subcutaneous administration of PEG-IFN β-1a or IFN β-1a to Rhesus monkeys resulted in an increase of serum neopterin concentrations, compared with pretreatment, confirming the in vivo biological activities of both test articles. In general, the neopterin concentration peaked at 24 to 32 h postdose in study 1, declined thereafter, and almost returned to baseline concentrations at 1 week postdose. It is noteworthy that despite the significant difference in drug exposure there were no discernable differences in neopterin profiles over the time course for all four treatment groups. The lack of higher neopterin response after PEGylation was reflected in the EC50 estimates from the PD model, where the EC50 of PEG-IFN β-1a was 21-fold higher than that of IFN β-1a. This result is consistent with previous observations for a 20-kDa mPEG-propionaldehyde-conjugated IFN β-1a (a predecessor of the current molecule), where no apparent differences were observed in neopterin response between the PEGylated and unmodified proteins, even with an approximately 10-fold difference in AUC (Pepinsky et al., 2001). Overall, the PD model well described the neopterin concentration-time profiles across test articles, routes, and doses (Fig. 4). The estimated elimination rate of neopterin loss was consistent with value from the literature (Table 3) (Mager et al., 2003, 2005). The variance seemed to be overestimated, which might be a result of small sample size (n = 4–5 for the full PD profiles for each stratified group). It is noteworthy that both N_{AUC,168h} and N_{peak} were lower in study 2 when PEG-IFN β-1a was administered subcutaneously at 10 μg/kg (1.1 MIU/kg) than those in study 1 at 10.8 μg/kg (1 MIU/kg) (Table 4). The two batches of materials in the two studies were comparable based on specification tests, including pH, osmolality, in vitro antiviral potency, peptide map, oxidation, and carbohydrate composition, using prespecified criteria. The difference is therefore likely to be attributable to interstudy variability, which might partly account for the overestimated variance in the PD model. Based on the estimated IC_{50} of 1.82 ng/ml, the

**TABLE 2**

Population PK model parameters (fixed and random effects), including percentage of relative S.E.

<table>
<thead>
<tr>
<th>Model Parameter Definition</th>
<th>IFN β-1a</th>
<th>PEG-IFN β-1a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimated Value</td>
<td>Relative S.E.</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Ka, SC, h⁻¹</td>
<td>Absorption rate after subcutaneous administration</td>
<td>1.37</td>
</tr>
<tr>
<td>Ka, IM, h⁻¹</td>
<td>Absorption rate after intramuscular administration</td>
<td>1.65</td>
</tr>
<tr>
<td>Vc, ml/kg</td>
<td>Central compartment volume of distribution</td>
<td>1610</td>
</tr>
<tr>
<td>Vp, ml/kg</td>
<td>Peripheral compartment volume of distribution</td>
<td>1170</td>
</tr>
<tr>
<td>CL, ml/kg/h</td>
<td>Clearance</td>
<td>314</td>
</tr>
<tr>
<td>Q, ml/kg/h</td>
<td>Intercompartmental clearance between central and peripheral compartment</td>
<td>67.6</td>
</tr>
<tr>
<td>F, SC</td>
<td>Relative bioavailability to intramuscular</td>
<td>0.781</td>
</tr>
<tr>
<td>F, IM</td>
<td>Relative bioavailability</td>
<td>1, Fixed</td>
</tr>
<tr>
<td>(\omega^\nu_{Vc})</td>
<td>Intersubject variance of Vc</td>
<td>0.127</td>
</tr>
<tr>
<td>(\omega^\nu_{CL})</td>
<td>Intersubject variance of CL</td>
<td>0</td>
</tr>
<tr>
<td>(\omega^\nu_{Q})</td>
<td>Intersubject variance of Q</td>
<td>0.487</td>
</tr>
<tr>
<td>cov(Vc, Q)</td>
<td>Covariance of Vc and Q</td>
<td>0.106</td>
</tr>
<tr>
<td>cov(CL, Q)</td>
<td>Covariance of CL and Q</td>
<td>0</td>
</tr>
<tr>
<td>SD</td>
<td>Coefficient of residual error</td>
<td>0.397</td>
</tr>
<tr>
<td>(\sigma^2)</td>
<td>Residual error variance</td>
<td>1, Fixed</td>
</tr>
</tbody>
</table>

SC, subcutaneous; IM, intramuscular; N.A., not applicable.
stimulatory effect will be inhibited by 92% at the peak neopterin concentration of 21 ng/ml (Table 3).

Dose and Route Effect on PK/PD of PEG-IFN β-1a in Rhesus Monkeys. In studies 2 and 3, PK was characterized over a dose range of 2 to 100 g/kg (0.22–11 MIU/kg). The mean drug concentration-time profiles of the subcutaneous groups from study 2 are shown in Fig. 5A. The PK profiles from study 3 after the first dose were similar to those in study 2 (data not shown). Overall, PEG-IFN β-1a showed linear kinetics, as shown by dose-proportional increases in AUC_{\text{inf}} and C_{\text{max}} and dose-independent $t_{1/2}$ and CL/F. The linear kinetics was further confirmed by decent linear model predictions (Fig. 3).

The $N_{\text{AUC,10h}}$ and $N_{\text{peak}}$ of neopterin increased with dose in a less than dose-proportional manner. With a 5-fold increase in PEG-IFN β-1a dose from 0.22 to 1.1 MIU/kg, the

### TABLE 3
Final population PD model parameters (fixed and random effects), including percentage of relative S.E.

<table>
<thead>
<tr>
<th>Model Parameter</th>
<th>Definition</th>
<th>IFN β-1a</th>
<th>PEG-IFN β-1a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Estimated Value</td>
<td>Relative S.E.</td>
</tr>
<tr>
<td>BSL, ng/ml</td>
<td>Baseline neopterin serum concentration</td>
<td>2.97</td>
<td>6</td>
</tr>
<tr>
<td>Loss, h⁻¹</td>
<td>First-order elimination rate of serum neopterin</td>
<td>0.0174</td>
<td>7</td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>Maximum stimulatory effect</td>
<td>198</td>
<td>31</td>
</tr>
<tr>
<td>MTT, h</td>
<td>Mean transit time in transit compartments</td>
<td>7.13</td>
<td>6</td>
</tr>
<tr>
<td>EC_{50}, pg/ml</td>
<td>Drug concentration to achieve 50% of $E_{\text{max}}$</td>
<td>2980</td>
<td>44</td>
</tr>
<tr>
<td>IC_{50}, ng/ml</td>
<td>Neopterin concentration to inhibit 50% of the stimulatory effect</td>
<td>1.82</td>
<td>29</td>
</tr>
<tr>
<td>$\sigma^2_{\text{BSL}}$</td>
<td>Intersubject variance of $V_C$</td>
<td>0.264</td>
<td>27</td>
</tr>
<tr>
<td>$\sigma^2_{\text{E_{max}}}$</td>
<td>Intersubject variance of $E_{\text{max}}$</td>
<td>0.185</td>
<td>31</td>
</tr>
<tr>
<td>Cov[BSL, $E_{\text{max}}$]</td>
<td>Covariance of BSL and $E_{\text{max}}$</td>
<td>-0.0859</td>
<td>38</td>
</tr>
<tr>
<td>S.D.</td>
<td>Coefficient of residual error</td>
<td>0.173</td>
<td>8</td>
</tr>
<tr>
<td>$\sigma^2$</td>
<td>Residual error variance</td>
<td>1, Fixed</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

N.A., not applicable.
was equivalent to 8, 40, and 400% of the EC50, corresponding to 7, 28, and 80% of the Emax, respectively, accounting at least partly for the less than dose-proportional response. In addition, the inhibitory feedback of neopterin concentration contributing a higher inhibitory effect. For instance, the Npeak from study 2 was equivalent to 6×, 8×, and 13× IC50 at 0.22, 1.1, and 11 MIU/kg s.c., respectively, corresponding to 85, 89, and 92% of inhibition of the stimulation by PEG-IFN β-1a.

The impact of route of administration on the PEG-IFN β-1a PK parameters was investigated in all three studies. The differences in AUCinf estimated by NCA were small (within ± 25% across the three studies) and no consistent trend was observed; however, the population F (subcutaneous) estimated by the PK model was 0.87 relative to F (intramuscular), which was fixed as 1, equivalent to a 13% lower AUCinf for the subcutaneous route. Given the observed %CV in NCA AUCinf (~30%), the difference was considered minimal. More profound differences were observed in the absorption kinetics. PEG-IFN β-1a was absorbed more quickly after intramuscular injection than after subcutaneous injection, resulting in an earlier Tmax (4.29 versus 14.6 h) and higher Cmax (4.79 versus 2.48 ng/ml/h), which was consistent across the three studies (Table 1). No consistent differences were identified between the intramuscular and subcutaneous routes in t1/2 or CL/F. From the modeling approach, the Ka (subcutaneous) was 5-fold lower than the Ka (intramuscular), corresponding to a 5-fold longer absorption half-life (7.8 versus 1.5 h) for the subcutaneous route. Inclusion of route as a covariate for CL, Vc, or Vp, did not improve model performance significantly, indicating a lack of impact by route on these parameters.

The effect of route on neopterin response was also evaluated. No apparent trend was identified with regard to route

### Table 4

Mean pharmacodynamic parameters of neopterin from noncompartmental analysis with S.D. shown in parentheses.

<table>
<thead>
<tr>
<th>Study</th>
<th>Test Article</th>
<th>Route</th>
<th>Dose</th>
<th>Dose Number</th>
<th>n</th>
<th>N_{AUC,168h}</th>
<th>N_{peak}</th>
<th>N_{E_{max}}</th>
<th>µg/kg; MIU/kg</th>
<th>hng/ml</th>
<th>hng/ml</th>
<th>h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IFN β-1a</td>
<td>IM</td>
<td>5; 1</td>
<td>1</td>
<td>5</td>
<td>1970 (689)</td>
<td>21.0</td>
<td>27.2</td>
<td>4.38</td>
<td>25.6</td>
<td>30.0</td>
<td>4.0</td>
</tr>
<tr>
<td>1</td>
<td>IFN β-1a</td>
<td>SC</td>
<td>5; 1</td>
<td>1</td>
<td>5</td>
<td>1680 (327)</td>
<td>21.4</td>
<td>24.0</td>
<td>0.0</td>
<td>28.8</td>
<td>30.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1</td>
<td>PEG-IFN β-1a</td>
<td>IM</td>
<td>10.8;1</td>
<td>1</td>
<td>5</td>
<td>2050 (266)</td>
<td>21.8</td>
<td>24.0</td>
<td>0.0</td>
<td>25.6</td>
<td>30.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1</td>
<td>PEG-IFN β-1a</td>
<td>SC</td>
<td>10.8;1</td>
<td>1</td>
<td>5</td>
<td>2050 (441)</td>
<td>20.2</td>
<td>24.0</td>
<td>0.0</td>
<td>28.8</td>
<td>30.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>PEG-IFN β-1a</td>
<td>SC</td>
<td>2; 0.22</td>
<td>1</td>
<td>4</td>
<td>1090 (539)</td>
<td>10.4</td>
<td>30.0</td>
<td>13.9</td>
<td>30.0</td>
<td>30.0</td>
<td>13.9</td>
</tr>
<tr>
<td>2</td>
<td>PEG-IFN β-1a</td>
<td>SC</td>
<td>10; 1.1</td>
<td>1</td>
<td>4</td>
<td>1560 (462)</td>
<td>14.8</td>
<td>24.0</td>
<td>0.0</td>
<td>4.0</td>
<td>4.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>PEG-IFN β-1a</td>
<td>SC</td>
<td>100; 11</td>
<td>1</td>
<td>4</td>
<td>2640 (573)</td>
<td>22.8</td>
<td>4.0</td>
<td>12.0</td>
<td>30.0</td>
<td>30.0</td>
<td>12.0</td>
</tr>
<tr>
<td>2</td>
<td>PEG-IFN β-1a</td>
<td>IM</td>
<td>100; 11</td>
<td>1</td>
<td>3*</td>
<td>3190 (625)</td>
<td>27.0</td>
<td>30.0</td>
<td>13.9</td>
<td>30.0</td>
<td>30.0</td>
<td>13.9</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>IM, SC</td>
<td>0</td>
<td>1</td>
<td>12</td>
<td>N.A.</td>
<td>5.02</td>
<td>24.0</td>
<td>0.0</td>
<td>24.0</td>
<td>24.0</td>
<td>0.0</td>
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<td>3</td>
<td>Control</td>
<td>IM, SC</td>
<td>0</td>
<td>3</td>
<td>12</td>
<td>N.A.</td>
<td>3.66</td>
<td>24.0</td>
<td>0.0</td>
<td>24.0</td>
<td>24.0</td>
<td>0.0</td>
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<tr>
<td>3</td>
<td>Control</td>
<td>IM, SC</td>
<td>0</td>
<td>5</td>
<td>12</td>
<td>N.A.</td>
<td>3.39</td>
<td>24.0</td>
<td>0.0</td>
<td>24.0</td>
<td>24.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>IM, SC</td>
<td>0</td>
<td>2; 0.22</td>
<td>1</td>
<td>8</td>
<td>N.A.</td>
<td>9.27</td>
<td>24.0</td>
<td>24.0</td>
<td>24.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>IM, SC</td>
<td>0</td>
<td>2; 0.22</td>
<td>5</td>
<td>8</td>
<td>N.A.</td>
<td>8.28</td>
<td>24.0</td>
<td>24.0</td>
<td>24.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>IM, SC</td>
<td>0</td>
<td>2; 0.22</td>
<td>1</td>
<td>8</td>
<td>N.A.</td>
<td>5.85</td>
<td>24.0</td>
<td>24.0</td>
<td>24.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>IM, SC</td>
<td>0</td>
<td>10; 1.1</td>
<td>3</td>
<td>8</td>
<td>N.A.</td>
<td>26.9</td>
<td>24.0</td>
<td>24.0</td>
<td>24.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>IM, SC</td>
<td>0</td>
<td>10; 1.1</td>
<td>5</td>
<td>8</td>
<td>N.A.</td>
<td>16.7</td>
<td>24.0</td>
<td>24.0</td>
<td>24.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>IM, SC</td>
<td>0</td>
<td>10; 1.1</td>
<td>1</td>
<td>8</td>
<td>N.A.</td>
<td>4.81</td>
<td>24.0</td>
<td>24.0</td>
<td>24.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

IM, intramuscular; SC, subcutaneous; N.A., not available.

* Data from one monkey were excluded because of unusually high predose and postdose peak concentration (25.8 and 100 ng/ml, respectively).

* One postdose sample was collected at 24 h as an approximation of Npeak after administration of doses 1, 3, and 5; hence, N_{AUC,168h} was not available and N_{E_{max}} was 24 h for all animals in study 3.
of administration and the magnitude of neopterin elevation. In study 1, the difference [(intramuscular − subcutaneous)/ subcutaneous%] was 0.2% in $N_{AUC_{168h}}$ and 8% in $N_{peak}$. In study 2, the differences were 21% in $N_{AUC_{168h}}$ and 19% in $E_{max}$. In study 3, neopterin concentration was measured at 24 h only for selected weeks; therefore, the parameters were not calculated. The percentage difference in neopterin concentration was −14% after the first dose before the immunogenic response. The route did not show significant impact on EC$_{50}$ or $E_{max}$ in the PD model. Overall, the effect of the route of administration on neopterin response was considered minimal.

**Immunogenicity and its Impact on PK/PD of PEG-IFN β-1a in Rhesus Monkeys.** Because IFN β-1a is immunogenic in Rhesus monkeys (Martin et al., 2002; Food and Drug Administration Toxicology Review of Rebif BLA, 1999, http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/ucm106138.pdf; Biogen Idec, data on file), antidrug antibodies were measured after single-dose administration (study 2) and after repeat-dose administration (study 3). In study 2, BABs and NABS were detected in 25 and 8% of the monkeys, respectively, at 14 days postdosing and in 50% and 50%, respectively, at 28 days postdosing. In study 3, BABs and NABS were detected in 85 and 35% of the PEG-IFN β-1a-treated-monkeys, respectively, after two doses; BABs and NABS were detected in 100 and 95% of the monkeys, respectively, after five doses (Fig. 6D).

The impact of immunogenicity on PK/PD of PEG-IFN β-1a was inferred from the repeat-dose toxicity study. The PEG-IFN β-1a concentration decreased significantly after multiple weekly doses, secondary to the development of antidrug antibodies (Fig. 6, A and B). Starting from the fourth dose, drug concentration became undetectable in 86% of the monkeys in the 2 μg/kg dose group and in 14% of the monkeys in the 10 μg/kg dose group. After the fifth dose, PEG-IFN β-1a was not detectable in 73% of the drug-treated monkeys. In the 11 monkeys that had detectable PEG-IFN β-1a after the fifth dose, the AUC was less than 1% (0.0009–0.98%) of the AUC from the first dose in nine monkeys, whereas the other two had AUC values that were 14.8 and 25% of the respective first dose.

The pharmacological response in Rhesus monkeys, as indicated by the serum neopterin concentration, was also affected by the immunogenicity of PEG-IFN β-1a. Administration of PEG-IFN β-1a to Rhesus monkeys initially induced the elevation of neopterin. However, the induction of neopterin decreased with successive doses (Fig. 6C). The mechanism by which the antidrug antibodies reduced the pharmacological response is probably caused by the bound antibody’s ability to prevent or diminish the interaction of the IFN
portion of the molecule with the type I IFN receptor, thereby reducing IFN-dependent signaling. However, we cannot rule out the possibility that an increased rate of drug clearance, mediated by the binding of antidrug antibodies, may also contribute to limiting exposure of the protein to its receptor and therefore reducing neopterin induction.

Safety of PEG-IFN β-1a in Rhesus Monkeys. Designed to support phase I clinical studies, the nonclinical good laboratory practice toxicology study evaluated the safety of PEG-IFN β-1a after repeat-dose subcutaneous or intramuscular administration for 5 weeks in Rhesus monkeys with a 4-week treatment-free recovery period. PEG-IFN β-1a was well tolerated in all dose groups and produced the expected pharmacological effects of transient increases in body temperature, increases in serum neopterin, and a transient decrease in peripheral blood lymphocytes. There were no PEG-IFN β-1a-related adverse effects on mortality, clinical observations, body weight, food consumption, local irritation, ophthalmology, heart rate, ECGs, respiratory rate, clinical pathology, urinalysis, or macroscopic or microscopic anatomic pathology at doses up to 100 μg/kg (subcutaneous or intramuscular) administered once weekly for 5 weeks.

Treatment with PEG-IFN β-1a resulted in nondose-dependent transient increases in body temperature of 1 to 3°F (Fig. 7) and a dose-dependent decrease in lymphocyte counts (Fig. 8). The decrease in lymphocyte counts was transient and evident only during week 1, with the exception of monkeys administered 100 μg/kg i.m., where the decrease in lymphocyte counts persisted through week 5, but not through week 6. The week 1 (8 h postdose) average lymphocyte counts were 1, 28, 33, 59, and 59% lower in monkeys administered vehicle control (subcutaneously and intramuscularly), 2 μg/kg s.c., 10 μg/kg s.c., 100 μg/kg s.c., and 100 μg/kg i.m. PEG-IFN β-1a, respectively, compared with lymphocyte counts 10 days before the first dose. Monkeys administered 100 μg/kg of PEG-IFN β-1a intramuscularly had lymphocyte counts (collected 4 h postdose) that were 64, 42, and 36% lower at weeks 3, 4, and 5, respectively, compared with the lymphocyte counts 10 days before the first dose. Thus, it seems that dosing of PEG-IFN β-1a via the intramuscular route perpetuates a reduction in lymphocyte counts during the dosing phase, with a noted recovery upon cessation of dosing. Because the effects on body temperature and lymphocyte counts were mild, reversible, and related to the expected pharmacological activity of type I IFNs, they were not considered adverse. There were no apparent sex-related differences with any of the findings. The no-observed-adverse-effect level from this study was 100 μg/kg (11 MIU/kg) for both subcutaneous and intramuscular routes, which was the highest dose tested.

Discussion

PEGylation is a method for extending the half-life of biological therapies, enhancing their in vivo efficacy, and enabling less frequent dosing (Bailon and Won, 2009). The mechanism underlying these effects is the increase in the apparent molecular size of the drug, which in turn reduces the glomerular filtration rate. The cutoff size for renal clearance is considered to be approximately the size of albumin (67 kDa). IFN β-1a has a molecular mass of ~25 kDa, and the kidney has been shown to be the major site of interferon degradation based on published animal studies, among which one study quantified the renal clearance contribution as more than 90% of total clearance in rats (Bino et al., 1982a,b; Bocci et al., 1984). The clearance pathway of interferon α by the kidneys is described as an initial filtration, followed by absorption by the proximal tubular cells, and subsequent catabolism in cellular lysosomes (Bocci et al., 1981, 1984). Incorporation of a single 20-kDa PEG resulted in an increase in the apparent Mr of the protein from ~25 to ~320 kDa as measured by size exclusion chromatography (Baker et al., 2006). The increase in the apparent mass compared with the unmodified protein results from a significant increase in the hydrodynamic volume. In addition to decreasing glomerular filtration rate, steric hindrance by the flexible PEG molecule could potentially protect the protein from protease degradation and antibody and receptor-mediated clearance mechanisms.

The N terminus of IFN β-1a serves as an attractive site for modification because it does not participate in receptor bind-
Using the same site-specific N-terminal PEGylation procedure, a similar PEG-IFN-β1a molecule has been synthesized previously with 20-kDa mPEG-propionaldehyde. Although 20-kDa mPEG-propionaldehyde-modified IFN-β1a has improved PK properties in mice, rats, and Rhesus monkeys and retained full in vitro antiviral activity (Pepinsky et al., 2001), direct side-by-side comparison of 20-kDa mPEG-propionaldehyde- and 20-kDa mPEG-O-2-methylpropionaldehyde-modified IFN-β1a has not been carried out. However, comparisons made to unmodified IFN-β1a in each case can be used to compare the two PEGylated molecules. Both PEGylated molecules showed 2- to 3-fold increase in t½ over unmodified IFN-β1a. The AUC increase over IFN-β1a was much greater (~40-fold) for the current molecule than for the previous molecule (~10-fold), but the comparison may not be relevant, because different bioanalytical methods (ELISA versus cytotoxic effect) were used to quantify serum drug concentrations. In terms of pharmacological response, both molecules produced similar neopterin profiles compared with unmodified IFN-β1a. Overall, the two molecules showed similar PK/PD properties in Rhesus monkeys. Compared with the PEG-IFN-β1a described here, PEGylated IFN-α-2b (PEG-Intron), a 12-kDa PEG conjugate, showed a t½ of 29.5 to 34.1 h after subcutaneous administration in cynomolgus monkeys and a 2- to 3-fold increase in dose-normalized AUC compared with the unmodified protein (Food and Drug Administration Preclinical Review of PEGINTRON BLA, 2000, http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplicationsTherapeuticBiologicApplications/ucm094485.pdf), whereas PEGylated IFN-α-2a (Pegasys), a 40-kDa PEG conjugate, provided a t½ of 143 to 186 h with AUC values not being available (Food and Drug Administration Preclinical Review of PEGASYS BLA, 2001, http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplicationsTherapeuticBiologicApplications/ucm094465.pdf). The differences in PK parameters may indicate the effect of the size of the conjugated PEG; however, comparison of values is limited by the different monkey species and assays used in each study.

The increase in AUCinf and Cmax observed for the PEG-IFN-β1a reported here did not translate into an increase in neopterin exposure. Similar results were reported for the 20-kDa mPEG-propionaldehyde-modified IFN-β1a (Pepinsky et al., 2001). Possible explanations proposed in that article included down-regulation of the type I IFN receptor or downstream signaling components, neopterin-producing cells being less accessible to PEG-IFN-β1a because of its limited tissue distribution or saturation of the biological response. The PK/PD model suggests that the stimulatory effect was indeed reduced approximately 1 day postdose. The reduced stimulatory effect could be explained by down-regulation of the type I IFN receptor (Pestka et al., 1987) and/or neopterin inhibitory feedback (Liberati et al., 1988). The latter approach was adopted as the mathematical solution, which linked an inhibitory effect to the neopterin concentration, because it provided more robust parameter estimates. Further evidence is necessary to distinguish between these two potential mechanisms. The saturation of the biological response at 1 MIU/kg is less likely, given the fact that the N AUC168h and Npeak increased further when the dose of PEG-IFN-β-1a was increased from 10 μg/kg (1.1 MIU/kg) to 100 μg/kg (11 MIU/kg) and the stimulatory effect in study 1 was ~60% of the Emax even at the Cmax. Indeed, although the PD parameters increased with dose, they trended toward saturation at 11 MIU/kg (100 μg/kg). For the subcutaneous route of administration, for which doses of 2, 10, and 100 μg/kg were tested (study 2), the neopterin dose-response curves indicated that for both NAUC168h and Npeak, the half-maximal saturation occurred at approximately 10 μg/kg. At this dose, the Cmax value was 19.5 ± 6.5 ng/ml, equating to a molar serum concentration of 9.7 × 10⁻¹⁰ ± 3.2 × 10⁻¹⁰ M. Although the affinity of PEG-IFN-β-1a for the Rhesus monkey type I IFN receptor is not known, assuming that the PEG moiety does not significantly affect receptor binding (the Kd for IFN-β-1a and PEG-IFN-β-1a binding to the isolated extracellular portion of the IFNAR2 chain of the human type I IFN receptor are the same; Biogen Idec, data on file), and the affinity of IFN-β-1a for the Rhesus monkey and human receptors is comparable, published data for the affinity of IFN-β for the human receptor can be used to estimate whether the nonlinear neopterin response is caused by receptor saturation or biological saturation. Because the affinity of IFN-β for the type I IFN receptor on the surface of human Daudi B cells is 3.2 × 10⁻¹⁰ M (range 0.9 to 11 × 10⁻¹⁰ M) (Cutrone and Langer, 1997), it suggests that the saturation of the neopterin response in Rhesus monkeys is caused by receptor saturation, i.e., 50% response at approximately the Kd. However, because the assumptions made above have yet to be verified, and we do not know whether the neopterin-producing cells are located within the vasculature, from which the Cmax concentration was measured, or outside of the vasculature, where the concentration is not known, we cannot rule out the possibility that the nonlinear neopterin response may be caused by saturation of biological responses downstream of the receptor. Indeed, IFN-β-dependent biological responses are known to saturate at ligand concentrations well below the Kd, i.e., at substoichiometric receptor occupancy. For example, Runkel et al. (2000) have shown that the half-maximal antiproliferative activity of IFN-β-1a on human Daudi B cells occurs at only 0.3% receptor occupancy.

It is noteworthy that these observations in Rhesus monkeys are in contrast to those in humans, where neopterin elevation was of longer duration after PEG-IFN-β-1a treatment than that after IFN-β-1a treatment at comparable doses (Hu et al., 2011). Furthermore, preliminary PK/PD modeling did not indicate reduction of the stimulatory effect in humans over a 1-week exposure (data not shown). This highlights the limitation of using an animal model to predict human responses. Because we are encouraged by the results from two phase 1 studies, the efficacy of PEG-IFN-β-1a is currently being tested in a multicenter, randomized, double-blind, placebo-controlled phase 3 clinical trial in patients with relapsing MS.

Human IFN-β-1a is immunogenic in monkeys (Martin et al., 2002; Food and Drug Administration Toxicology Review of Rebib BLA, 1999, http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplicationsTherapeuticBiologicApplications/ucm106138.pdf; Biogen Idec, data on file). Even though PEGylation has been shown to reduce immunogenicity of some proteins in humans (http://www.accessdata.fda.gov/
drugatfda_docs/label/2006/103145s5060LBL.pdf; http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/103964s5147, %20103964s5163lbl.pdf), PEG-IFN 1a remained immunogenic in Rhesus monkeys, especially in the repeat-dose study, in which almost all monkeys were BAB- and NAB-positive after five weekly doses. Consequently, the PEG-IFN 1a concentration decreased dramatically, and the neopterin response diminished almost completely by the end of the study. However, the immunogenicity of PEG-IFN 1a in monkeys is not predictive of the human response. When PEG-IFN 1a was tested in humans, no subject developed NABs after single- or multiple-dose PEG-IFN 1a treatment (Baker et al., 2010; Hu et al., 2011). This observation is consistent with IFN 1a, of which the immunogenicity rate was 2 to 5% in a study in patients with MS (Panitch et al., 2002; http://www.accessdata.fda.gov/drugsatfda_docs/label/2007/103628s5115lbl.pdf), in contrast to 100% in studies in Rhesus monkeys (Biogen Idec, data on file). The immunogenicity of PEG-IFN 1a over long-term treatment is being tested in the ongoing phase 3 study.

Pharmacology and safety of unmodified IFN 1a have been evaluated in more than 10 studies in Rhesus monkeys, including both single- and repeat-dose evaluations (Biogen Idec, data on file). Monkeys developed NABs to IFN 1a after approximately 2 weeks of treatment; for this reason, chronic repeat dose toxicity testing was not feasible. No mortalities and no significant signs of toxicity were observed in the treated animals up to the highest dose of IFN 1a tested (50 μg/kg s.c.). Elevated body temperature, reduced food consumption, decreased platelet counts, and decreased serum albumin and calcium concentrations were observed. No respiratory, cardiovascular, or acute allergic reactions were evident. No overt effects on the central nervous system were noted in any of the treated animals. Compared with historic data, the safety profile of PEG-IFN 1a in Rhesus monkeys is consistent with that of IFN 1a. The responses, including a slight increase in body temperature and decreases in lymphocyte counts, are anticipated effects in response to IFN 1a treatment and were shown to be reversible after the treatment-free period. Similar to IFN 1a, monkeys also developed NABs within 2 weeks of repeat treatment, resulting in reduced or no exposure in 5 weeks and rendering chronic repeat-dose toxicity testing with PEG-IFN 1a not feasible. PEGylating IFN 1a did not result in any new toxicities in Rhesus monkeys, even with the changes in PK. It is noteworthy that other effects seen upon treatment of monkeys with IFN 1a included decreased platelets, transient elevation of hepatic transaminases, transient decreases in serum total protein and albumin concentrations, and local irritation and/or inflammation, none of which were seen with PEG-IFN 1a treatment in the toxicity study of PEG-IFN 1a.

In summary, in Rhesus monkeys PEG-IFN 1a showed greater exposure, longer half-life, slower clearance, and reduced volume of distribution compared with IFN 1a. Consistent with the pharmacology of type I IFNs, PEG-IFN 1a induced neopterin elevation and caused transient body temperature increase and reversible lymphocyte count decrease. However, at comparable 1 MIU/kg doses, PEG-IFN 1a did not result in enhancement of neopterin induction compared with the unmodified protein, an observation that contrasts to the situation in humans where the improved PK properties of the molecule translated into improved PD responses. There were no drug-related adverse effects at doses up to 100 μg/kg (11 MIU/kg s.c. or i.m.) administered once weekly for 5 weeks, and the no-observed-adverse-effect level was determined to be 100 μg/kg. These studies were used to successfully enable two phase 1 clinical trials of PEG-IFN 1a in healthy subjects (Hu et al., 2011); data from which in turn enabled a phase 3 study in patients with relapsing MS.

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References


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