Pharmaceutical Research, Vol. 22, No. 1, January 2005 (© 2005)
DOI: 10.1007/s11095-004-9009-z

Research Paper

Pharmacokinetics and Pharmacodynamics of PEGylated IFN-β 1a Following Subcutaneous Administration in Monkeys

Donald E. Mager,[1] Berend Neuteboom,[2] and William J. Jusko[1,3]

Received June 10, 2004; accepted October 12, 2004

Purpose. To characterize the pharmacokinetic/pharmacodynamic (PK/PD) properties of a new polyethylene glycol (PEG) conjugate formulation of interferon (IFN)-β 1a following subcutaneous (SC) administration in monkeys.
Methods. Single SC injections of 0.3, 1, and 3 million international units (MIU)/kg of PEG-IFN-β 1a were administered to 3 groups of cynomolgus monkeys (n = 4 each). Plasma concentrations of drug and neopterin, a classic biomarker for IFN-β PD, were measured at various time-points after dosing. PK/PD profiles were described by noncompartmental methods and pooled data by an integrated mathematical model, where fixed and delayed concentration-time profiles were used as driving functions in an indirect stimulatory response model.
Results. PEG-IFN-β 1a was rapidly absorbed, with peak concentrations observed at about 4–5 h. Compared to previous identical SC doses of IFN-β 1a, administration of 1 and 3 MIU/kg of pegylated drug resulted in 27- and 16-fold increases in area under the concentration-time curves. Neopterin concentrations followed a typical dose-dependent biphasic pattern. Pooled PD profiles were well-described by the PK/PD model, and the neopterin elimination rate (0.0190 h^{-1}) is consistent with previous estimates.
Conclusions. The PEG-modification of IFN-β 1a provides enhanced drug exposure and similar pharmacodynamics of neopterin compared to the unmodified formulation.

KEY WORDS: interferon-beta 1a; neopterin; pharmacodynamics; pharmacokinetics; polyethylene glycol.

INTRODUCTION

A common difficulty associated with the use of proteins as therapeutic agents is rapid systemic clearance. For example, many cytokines are rapidly cleared from the circulation by means of receptor-mediated endocytosis (1). During the past two decades, protein conjugation with polyethylene glycol (PEG) has shown promise in overcoming this complication (2). Such a chemical modification usually causes a reduction in protein recognition by elimination mechanisms and can have considerable effects on the pharmacokinetics (PK) and pharmacodynamics (PD) of proteins (3).

Interferon (IFN)-β 1a is a therapeutic cytokine currently indicated for the treatment of multiple sclerosis and exhibits a relatively short plasma half-life. A PEG-modified form of IFN-β 1a has been reported (4), and was shown to provide enhanced plasma pharmacokinetic profiles in monkeys, rats, and mice. The *in vivo* pharmacological response to PEG- and unmodified-IFN-β 1a exposure was evaluated from drug-induced increases in plasma neopterin concentrations in monkeys, revealing comparable pharmacodynamics or preserved bioactivity between formulations. Here we provide further evidence of the improved PK and conserved PD properties of a novel PEG-IFN-β 1a conjugate in monkeys, along with a minimal model to characterize the PK/PD profiles resulting from the administration of single ascending subcutaneous (SC) doses.

METHODS

Animals

This study was conducted in adherence with the "Principles of Laboratory Animal Care" (NIH Publication No. 85-23, revised in 1985). Twelve *Macaca fascicularis* (Cynomol-

[1] Department of Pharmaceutical Sciences, University at Buffalo, State University of New York, Buffalo, New York 14260, USA.
[2] Serono Pre-Clinical Development Site, Istituto di Ricerche Biomediche "Antoine Marxer"-RBM SpA, 10010 Colleretto Giacosa (Torino), Italy.
[3] To whom correspondence should be addressed. (e-mail: wjjusko@buffalo.edu)

ABBREVIATIONS: A_p, amount of drug in central compartment; A_{sc}, amount of drug in SC administration site; A_t, amount of drug in non-specific binding compartment; k', first-order rate constant of drug absorption to and elimination from central compartment; k_{12}, first-order rate constant of drug distribution from central to nonspecific binding compartment; k_{21}, first-order rate constant of drug distribution from nonspecific binding to central compartment; k_{in}, zero-order rate constant of neopterin production; k_{out}, first-order rate constant of neopterin elimination; N, neopterin plasma concentration; N_0, baseline or time-zero neopterin concentration; SC_{50}, drug concentration producing 50% of S_{max}; S_{max}, capacity factor for drug stimulation of k_{in}; τ, pharmacodynamic time-lag; V/F, volume of distribution of PEG-IFN-β 1a corrected for bioavailability.

gus) monkeys were clearly identified and received a standardized diet during a period of acclimation. Animals were in good health at the time of the study.

Experimental

Three groups of 4 monkeys received single SC bolus injections of 0.3, 1, or 3 million international units (MIU)/kg of a 40-kDa PEG-IFN-β conjugate, in which a branched PEG molecule comprising two 20-kDa moieties is bound to residue Cys-17 of IFN-β 1a. Following drug administration, blood samples were collected and plasma drug concentrations were determined at 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, and 120 h using a commercially available ELISA kit (Toray Industries, Tokyo, Japan) validated for monkey plasma (lower limit of quantification was 10 IU/ml). Plasma neopterin concentrations were measured at 0, 6, 12, 24, 48, 96, 120, 168, 216, 264, and 336 h after dosing using a radioimmunoassay kit (ICN Biomedicals, Costa Mesa, CA, USA) validated for monkey plasma (quantification limit was 0.5 ng/ml).

PK/PD Model and Data Analysis

Individual drug concentration-time profiles were first analyzed using standard noncompartmental techniques (WinNonlin v2.1, Pharsight Corp., Apex, NC, USA) to determine whether drug exposure was proportional to dose (linear pharmacokinetics). An integrated PK/PD model for the SC administration of PEG-IFN-β 1a is shown in Fig. 1. The time-course of pooled drug concentrations was modeled first according to the following system of differential equations:

$$\frac{dA_{SC}}{dt} = -k' \cdot A_{SC} \tag{1}$$

$$\frac{dA_p}{dt} = k' \cdot A_{SC} - (k' + k_{12}) \cdot A_p + k_{21} \cdot A_t \tag{2}$$

$$\frac{dA_t}{dt} = k_{12} \cdot A_p - k_{21} \cdot A_t \tag{3}$$

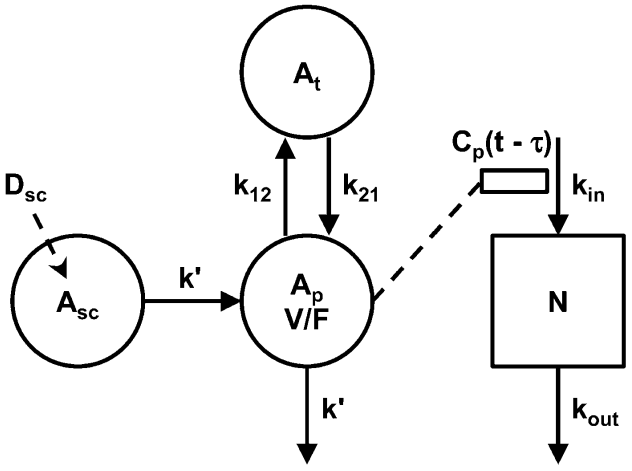

Fig. 1. Minimal PK/PD model of PEG-IFN-β 1a following single ascending SC doses in monkeys. The PK component resembles a standard linear two-compartment model with a single first-order rate constant for absorption to and elimination from the central compartment (k'). The PD model is a modified stimulatory indirect response model (5), where the driving function (PK) is delayed by a time-lag parameter (τ).

where the symbols are defined in the abbreviations section and drug concentrations (C_p) are set equal to $A_p/(V/F)$. Reported concentrations just below the quantification limit were retained in the analysis. The unknown parameters estimated from model fitting were k', k_{12}, k_{21}, and V/F. The initial condition of Eq. 1 is the SC dose (D_{sc}), whereas the initial conditions of Eqs. 2 and 3 are zero.

The pharmacodynamic component of the model is an indirect stimulatory response model (5) driven by fixed pharmacokinetic profiles (Eqs. 1–3), delayed in time by a time-lag parameter (τ). Pooled plasma neopterin concentrations (N) were thus described using the following equation:

$$\frac{dN}{dt} = k_{in} \cdot S(t) - k_{out} \cdot N \tag{4}$$

where the stimulatory function is given by,

$$S(t) = 1 + \frac{S_{max} \cdot C_p(t-\tau)}{SC_{50} + C_p(t-\tau)} \tag{5}$$

and parameter symbols are defined in the Abbreviations. The initial condition of Eq. 4 (N_0) was assumed to be stationary (i.e., $k_{in} = N_0 \cdot k_{out}$), leaving k_{out}, S_{max}, SC_{50}, N_0, and τ as unknown parameters to be estimated.

All PK/PD parameters were estimated by nonlinear regression analysis using the maximum likelihood estimator in ADAPT II (6). Methods of obtaining initial parameter values have been described elsewhere (7). A standard variance model was implemented as defined by:

$$VAR(C_p, N) = \sigma_1^2 \cdot Y^{\sigma_2} \tag{6}$$

where σ_i are the variance model parameters (separate parameters were used for PK and PD measures) and Y represents a matrix of model predicted values.

RESULTS AND DISCUSSION

The PK/PD properties of IFN-β 1a following intravenous (IV) and SC administration in monkeys have been recently reported (8). The unmodified formulation resulted in significant nonlinear pharmacokinetics, with full characterization requiring an integrated PK/PD model based upon pharmacological target-mediated drug disposition (9). Although SC administration of 1.0 and 3.0 MIU/kg provided prolonged exposure of IFN-β 1a and similar effects on neopterin as compared to similar IV doses, total drug exposure was lower (incomplete bioavailability) and plasma concentrations were below the quantification limit after 48 h.

In this study, the PK/PD properties of SC administered 40 kDa-PEG-IFN-β 1a were evaluated in monkeys, typically used to assess the *in vivo* activity of this drug owing to their ability to respond to human IFN-β 1a (4,8). The pooled concentration-time profiles of PEG-IFN-β 1a are shown in Fig. 2 (top panel) and the mean parameters from the noncompartmental analysis are listed in Table I. After SC administration, PEG-IFN-β 1a was rapidly absorbed with peak concentrations occurring around 4 to 5 h. The terminal elimination phase of the 0.3 MIU/kg dose appeared monoexponential, whereas concentrations from the 1.0 and 3.0 MIU/kg doses decreased in a poly-exponential manner. However, plasma drug concentrations declined below the quanti-

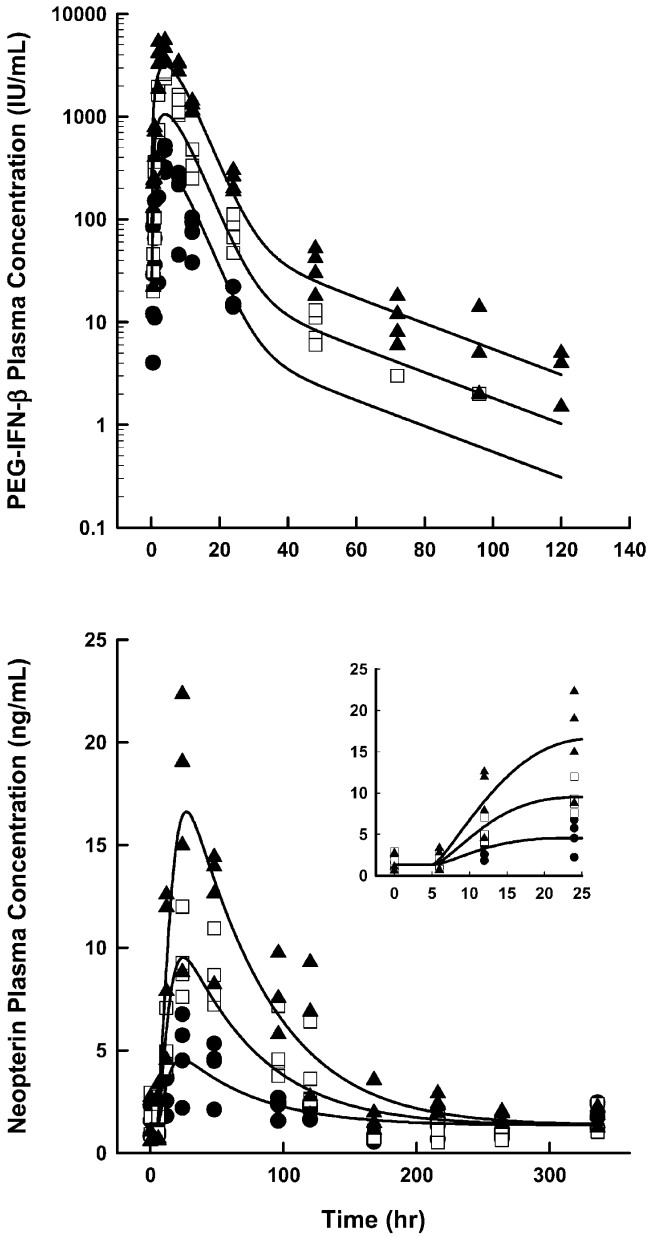

Fig. 2. Time course of pooled PEG-IFN-β 1a (top) and neopterin (bottom) concentrations following single SC doses of 0.3 (●), 1.0 (□), and 3.0 (▲) MIU/kg in monkeys. Inset graph in the bottom panel shows the first 25 h of neopterin concentrations. Symbols are measured concentrations, and lines represent model-fitted profiles.

fication limit sooner for the lowest dose, potentially masking the terminal phase and resulting in under-estimated areas under the concentration-time curves (AUC) and overestimated clearance values. Although total systemic clearance seemed higher for the lowest dose, differences in clearance values were not statistically significant (one-way ANOVA, $p = 0.121$). In contrast to unmodified IFN-β 1a, calculated AUC values were linearly related to dose ($r = 0.98$, $p < 0.0001$), suggesting that the pharmacokinetics of PEG-IFN-β 1a are linear in this dosage range. Compared to the same SC doses of IFN-β 1a (8), 1.0 and 3.0 MIU/kg of PEG-IFN-β 1a yielded approximately 27- and 16-fold increases in AUC values, along with 54- and 13-fold higher maximum plasma drug concentrations. Pepinsky *et al.* similarly reported a 9-fold increase in AUC and a 4-fold increase in C_{max} values, comparing 1.0 MIU/kg SC doses of unmodified- and PEG-IFN-β 1a (4). However, comparisons with this study must be made cautiously as a different PEG-conjugate was used (N-terminally linked 20 kDa PEG moiety). Furthermore, drug concentrations were determined using an antiviral bioassay, whereas a more specific immunoassay was used in the present analysis.

Pharmacokinetic profiles were reasonably described using a linear two-compartment model; however, peak concentrations for the 1.0 MIU/kg dose were slightly underpredicted (Fig. 2, top panel). A model with separate first-order absorption and elimination rates was tried, but these were replaced with a single rate constant, as these values were routinely estimated to be similar. This allowed for a reduction in the number of model parameters and improved the identifiability and precision of their estimation (Table II). Interestingly, a single absorption and elimination rate constant was also used with a one-compartment open model to describe the PK profiles of IFN-α 2a and PEG-IFN-α 2a after SC dosing in healthy adult male subjects (10).

Binding of IFN-β with its cell surface receptor initiates a cascade of intracellular events resulting in the induction of several biomarkers including neopterin and β2-microglobulin plasma concentrations, and intracellular 2′,5′-oligodenylate synthetase activity. More specifically, IFN-β is thought to stimulate GTP-cyclohydrolase I, which catalyzes the conversion of GTP to a neopterin precursor (neopterin triphosphate). Once formed, neopterin is eliminated primarily via renal excretion. These molecular processes can take time to manifest and form the basis for the approximately 5 to 6 h time-lag observed prior to the increase in neopterin concentrations. The time-course of pooled plasma neopterin concentrations following SC administration of PEG-IFN-β 1a is shown in Fig. 2 (bottom panel). After the time-lag, neopterin concentrations increase to peak values around 24 h after dosing and then gradually return to baseline.

Pooled neopterin pharmacodynamic profiles were well characterized by the PK/PD model (Fig. 2, bottom panel) and the estimated pharmacodynamic parameters are listed in Table II. The estimated elimination rate constant for neopterin ($k_{out} = 0.0190\ h^{-1}$) is in agreement with our previous estimate of $0.0184\ h^{-1}$ (8). A stimulatory indirect response model has been applied previously to describe the induction of MX protein by IFN-α 2a and a pegylated derivative (10), which is also initiated by drug binding to the common IFN-α/β receptor. However, the time-lag prior to the onset of effect required a modification to this standard indirect response model. Our previous pharmacodynamic model for neopterin induction by unmodified IFN-β 1a included a transduction compartment and a precursor-dependent indirect response model to account for the onset delay, which has a mechanistic basis (8). These more complex models were applied to the current data set but were not supported by standard model fitting criteria (e.g., additional rate constants in these models were not readily identifiable). A pharmacodynamic time-lag parameter was thus used as a substitute for precursor or transduction compartments, providing a simplified model for which good precision on parameter estimates was achieved (Table II). The concept of utilizing delayed drug concentrations as a pharmacodynamic driving function has been described for modeling the antiviral effects of PEG-

Table I. Noncompartmental Pharmacokinetic Parameters of PEG-IFN-β 1a in Monkeys

Dose (MIU/kg)	T_{max} (h)	C_{max} (IU/ml · 10^3)	$T_{1/2,\lambda z}$ (h)	AUC (IU · h/ml · 10^3)	CL/F (ml · h^{-1} · kg^{-1})	MRT (h)
0.3	4	0.400	3.89	3.13	121	7.23
	—[a]	(0.114)	(0.82)	(1.39)	(78)	(0.86)
1.0	4	2.59	9.56	19.8	51.1	8.15
	—[a]	(0.17)	(5.52)	(2.5)	(6.6)	(1.20)
3.0	5	4.32	21.0	47.3	63.9	10.2
	(4–8)	(1.01)	(7.1)	(5.3)	(6.4)	(1.4)

Values are reported as the mean (SD) of individual estimates (n = 4 each), except for T_{max}, where median (range) values are given. T_{max} and C_{max}, time and value of maximum plasma concentration; $T_{1/2,\lambda z}$, terminal elimination half-life; AUC; area under plasma concentration-time curve extrapolated to infinity; CL/F, total systemic clearance corrected for bioavailability; MRT, mean residence time.
[a] Values were identical for all animals.

IFN-α 2b (11). Although typically requiring the use of delay-differential equations, such a time-lag can be readily achieved by delaying the timing of the dosing event in ADAPT (6). The time-lag parameter (τ) was estimated to be 5.05 h and corresponds with visual inspection of the dynamic profiles from both formulations (see Fig. 2 and Ref. 8), suggesting that PEG-modification does not alter the temporal aspects of either signal transduction (12) or functioning of a precursor compartment (8). The relatively large stimulatory capacity factor (S_{max} = 72.4) appears to be countered by a large SC_{50} value (relative to plasma drug concentrations), resulting in net pharmacological effects that are similar to those produced by unmodified IFN-β 1a (8). Similar findings have been reported when comparing single doses of unmodified and pegylated IFN-β 1a (4), IFN-α 2a (10), and IFN-α 2b (13). Although decreased potential for immunogenicity may exist for use in humans, antibody formation was observed in monkeys (data not shown) and has also been reported for other PEG-IFN-β 1a conjugates (4).

In summary, the PEG-modification of IFN-β 1a provides enhanced drug exposure and comparable pharmacodynamics. An indirect response PK/PD model driven by delayed drug concentrations was proposed and well characterized the effects of the drug following single ascending doses in monkeys. Further studies are needed to ascertain whether PEG-IFN-β 1a provides any clinical therapeutic advantages over existing drug formulations.

Table II. Estimated PK/PD Model Parameters of PEG-IFN-β 1a in Monkeys

Parameter (units)	Estimate	CV%
Pharmacokinetics		
k_{12} (h^{-1})	0.0162	20
k_{21} (h^{-1})	0.0311	15
k' (h^{-1})	0.240	3.3
V/F (ml/kg)	338	6.9
Pharmacodynamics		
S_{max}	72.4	25
SC_{50} (IU/ml · 10^3)	1.32	38
k_{out} (h^{-1})	0.0190	8.8
N_0 (ng/ml)	1.34	7.8
τ (h)	5.05	7.2

Pooled PK and PD data were modeled separately as described in text. CV% values are of the model estimates and unrelated to inter-animal variability.

ACKNOWLEDGMENTS

We would like to thank Dr. Alain Munafo for carefully reviewing this manuscript. This study was supported by Serono International, Grant No. GM57980 from the National Institutes of Health (to W.J.J.), and a Pre-doctoral Fellowship from the American Foundation for Pharmaceutical Education (to D.E.M.).

REFERENCES

1. Y. Sugiyama and M. Hanano. Receptor-mediated transport of peptide hormones and its importance in the overall hormone disposition in the body. *Pharm. Res.* **6**:192–202 (1989).
2. J. M. Harris and R. B. Chess. Effect of pegylation on pharmaceuticals. *Nat. Rev. Drug Discov.* **2**:214–221 (2003).
3. R. Mehvar. Modulation of the pharmacokinetics and pharmacodynamics of proteins by polyethylene glycol conjugation. *J. Pharm. Pharm. Sci.* **3**:125–136 (2000).
4. R. B. Pepinsky, D. J. LePage, A. Gill, A. Chakraborty, S. Vaidyanathan, M. Green, D. P. Baker, E. Whalley, P. S. Hochman, and P. Martin. Improved pharmacokinetic properties of a polyethylene glycol-modified form of interferon-beta-1a with preserved in vitro bioactivity. *J. Pharmacol. Exp. Ther.* **297**:1059–1066 (2001).
5. N. L. Dayneka, V. Garg, and W. J. Jusko. Comparison of four basic models of indirect pharmacodynamic responses. *J. Pharmacokinet. Biopharm.* **21**:457–478 (1993).
6. D. Z. D'Argenio and A. Schumitzky. *ADAPT II User's Guide*, Biomedical Simulations Resource, Los Angeles, 1997.
7. J. Gabrielsson and D. Weiner. *Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications*, Swedish Pharmaceutical Press, Stockholm, 1997.
8. D. E. Mager, B. Neuteboom, C. Efthymiopoulos, A. Munafo, and W. J. Jusko. Receptor-mediated pharmacokinetics and pharmacodynamics of interferon-β 1a in monkeys. *J. Pharmacol. Exp. Ther.* **306**:262–270 (2003).
9. G. Levy. Pharmacologic target-mediated drug disposition. *Clin. Pharmacol. Ther.* **56**:248–252 (1994).
10. K. Nieforth, R. Nadeau, I. Patel, and D. Mould. Use of an indirect pharmacodynamic stimulation model of MX protein induction to compare in vivo activity of interferon alfa-2a and a polyethylene glycol-modified derivative in healthy subjects. *Clin. Pharmacol. Ther.* **59**:636–646 (1996).
11. K. A. Powers, N. M. Dixit, R. M. Ribeiro, P. Golia, A. H. Talal, and A. S. Perelson. Modeling viral and drug kinetics: hepatitis C virus treatment with pegylated interferon alfa-2b. *Semin. Liver Dis.* **23**:13–18 (2003).
12. D. E. Mager and W. J. Jusko. Pharmacodynamic modeling of time-dependent transduction systems. *Clin. Pharmacol. Ther.* **70**:210–216 (2001).
13. P. Glue, J. W. Fang, R. Rouzier-Panis, C. Raffanel, R. Sabo, S. K. Gupta, M. Salfi, and S. Jacobs. Pegylated interferon-alpha2b: pharmacokinetics, pharmacodynamics, safety, and preliminary efficacy data. Hepatitis C Intervention Therapy Group. *Clin. Pharmacol. Ther.* **68**:556–567 (2000).