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

Interferon alfa-2a was chemically modified by the covalent attachment of a polyethylene glycol (PEG) moiety to enhance its circulating half-life and to reduce its immunogenicity. A comparative evaluation of the pharmacokinetics of the PEG-modified interferon alfa-2a showed a greater than twofold increase in the circulating half-life as a result of this chemical modification. An indirect physiologic response model was developed to characterize the time course of the MX protein response after subcutaneous administration of single ascending doses of either interferon alfa-2a or PEG–interferon alfa-2a in healthy volunteers. Analysis of the pharmacokinetic-pharmacodynamic relationship suggested that the PEG-modified interferon alfa-2a could not be administered less than twice weekly and therefore offered little therapeutic advantage over its unmodified counterpart, which is administered three times weekly. These results were consistent with findings in phase II trials. This study substantiates the usefulness of pharmacodynamic modeling as a tool for the development of dose recommendations and for the early selection of drug candidates in the drug development process. (Clin Pharmacol Ther 1996;59:636-46.)

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Interferons were first discovered as a result of their antiviral activity.[1] They also exert a wide variety of pleiotropic effects, including the inhibition of cell growth, antitumor activity, involvement in hematopoiesis, and regulatory effects on cellular and humoral immune response.[2,3] Interferons exert their activity through interactions with specific cell surface receptors, resulting in either induction of cellular processes or enhancement of the transcription of specific genes.[4] This activity can be monitored by assaying for the presence of specific gene products or markers after the administration of interferon. For example, the decrease in serum tryptophan observed after a dose of interferon is indicative of increased activity of indoleamine dioxygenase, which is an interferon regulated protein thought to play a role in antiproliferative activity.[5]

There are numerous other measurable responses to interferon, including the induction of MX protein production.[6] MX protein is a 78 kD cytoplasmic protein that is selectively induced by type I interferons. MX proteins exert a plethora of effects on basic cellular functions involved in viral replication, thus conferring cellular immunity to specific viral infections.[7,8] MX proteins are thought to interact with guanosine 5′-triphosphate-dependent cellular processes, including microtubule formation and mitosis, because of their structural homology with other related proteins.[9] MX proteins also function as a minor histocompatibility antigen.[10] The concurrent implication is that MX proteins may have inherent antiproliferative activity. MX protein is an interferon regulated protein, and type I interferons are the only known influence on its production.[7,9] Furthermore, reproducible dose-related increases in MX protein concentration after interferon administration have been documented in the literature.[11,12] These characteristics

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make MX protein a useful pharmacodynamic probe for monitoring and comparison of the in vivo activity of interferon and new interferon preparations, despite the fact that MX protein has never been validated as a surrogate marker for predicting clinical response to interferon.

The administration of exogenous interferon elicits the same responses in vivo as endogenous interferon, which has led to the approval and use of interferon as a therapeutic agent for the treatment of several viral infections and malignancies.[13] However, interferon has several shortcomings as a therapeutic agent. Interferon is a protein and must therefore be administered parenterally. Furthermore, frequent dosing is necessary to maintain therapeutic concentrations. Recombinant forms of interferon can also be antigenic, and clinical response in patients ceases with the formation of neutralizing antibodies.

Monomethoxypolyethylene glycols (PEG) are nonimmunogenic, linear amphoteric polymers of varying molecular weight that can be covalently attached to proteins.[14] Chemical modification of certain therapeutic proteins with PEG has resulted in increased half-lives and reduced immunogenicity for these agents, with associated diminished specific activity (i.e., diminished activity per mass unit of protein).[14,15] The properties of the modified protein are dependent on both the average molecular weight and the number of PEGs attached.[16,17] Thus it was thought that modification of recombinant interferon with PEG would result in a novel therapeutic agent with less immunogenicity and a longer circulating half-life.

Although the administration of interferon as a therapeutic agent can have a palliative effect on certain diseases, dose regimens for many of the clinical indications remain largely empirical. The pharmacokinetics of interferons have been described in the literature[18,19]; however, characterization of the pharmacodynamics of interferon has generally been limited to descriptive analysis. Examination of the concentration-response relationship of interferon would therefore provide useful information to improve existing dose regimens and also to guide the development strategy of chemically modified interferons. Therefore the purpose of this analysis was to develop a pharmacokinetic-pharmacodynamic model to predict the biological activity of interferon alfa-2a and PEG–interferon alfa-2a as measured by human MX protein, with the expectation that reliable dosage recommendations could be made for future therapeutic trials on the basis of relative biological activity of interferon alfa-2a and PEG–interferon alfa-2a.

METHODS

Protocol. This was a double-blind, randomized, single ascending dose study performed on 45 healthy adult male subjects who ranged in age from 20 to 44 years and who weighed from 65 to 102 kg. Four dose groups each of interferon alfa-2a and PEG–interferon alfa-2a were studied: 3, 6, 9, and 18 million international units (MIU). There were nine planned subjects in each dose group; three subjects per group were randomized to received interferon alfa-2a and six subjects per group were randomized to received PEG–interferon alfa-2a; however, the 3 MIU dose group was retested in an additional group for technical reasons. Subjects were admitted to the study unit on the evening before test drug administration, where they remained for the duration of the study. An absolute fast (except for water) was maintained for 12 hours before the start of the study. The study was approved by the Institutional Review Board at the Newark Beth Israel Medical Center, Newark, N.J., and each subject gave written informed consent before enrollment. Interferon alfa-2a or PEG–interferon alfa-2a was administered in the morning as a single dose by subcutaneous injection into the upper arm. In anticipation of the febrile response associated with both compounds, 2 × 325 mg acetaminophen tablets were administered concomitantly with the dose and then every 4 hours thereafter for 24 hours. Blood samples were obtained by direct venipuncture in the arm contralateral to the injection site. Blood samples (~5 ml) for the determination of interferon alfa-2a and PEG–interferon alfa-2a levels were obtained immediately before administration (0 hours) and at 1, 1½, 3, 6, 12, 24, 48, 72, 96, and 120 hours. Blood samples (~15 ml) for the determination of MX protein concentrations were obtained at 0, 1, 3, 6, 12, 24, 48, 72, 96, 132, 168, 204, 240, and 276 hours.

Interferon alfa-2a and PEG–interferon alfa-2a assay. Serum levels of interferon alfa-2a and PEG–interferon alfa-2a were measured by an enzyme immunoassay.[20] Samples and standards were incubated in a single step with mouse anti-interferon monoclonal antibody coated beads and mouse anti-interferon monoclonal antibody peroxidase conjugate. The analyte was captured and labeled simultaneously. Beads were then washed and incubated with enzyme substrate. The color intensity developed by the enzyme was proportional to the amount of enzyme bound. After the enzymatic reaction was stopped, absorbance was read at 492 nm. Sample values were determined against a standard curve with use of a 200 ml serum sample. The lower

detection limit for this assay was 5 units/ml. The interassay precision was 4.8% for interferon alfa-2a and 5.8% for PEG–interferon alfa-2a. All serum levels of both interferon alfa-2a and PEG–interferon alfa-2a are reported in units per milliliter.

MX protein assay. MX protein assays were performed by Anawa Laboratories AG, Wangen Zürich, Switzerland. An immunochemiluminometric magnetic separation assay was selected as the method of measurement of MX protein.[21] This assay required heparinized, lysed, and denatured whole blood samples and used an acridinium ester-labeled antibody to specifically capture protein from the sample. A second polymagnetic particle conjugated antibody, also specific for the MX protein, was added subsequently. After an incubation period and washing steps with polymagnetic particle, the conjugated antibodies were separated from the sample with a magnetic box separator. This device allowed repeated washings of the double antibody MX protein sandwich while the magnetic particles were held in place in the tube. Finally, deionized water was added to the MX protein antibody sandwich in the tube and the chemiluminescence of the sample was measured with use of a luminometer. Relative light units were plotted versus MX protein concentration to form the standard curve, and the MX concentration from patient samples were assessed against the standard. The mean intraassay variability was 14.8%. The interassay variability was 25%. The lower detection limit of this assay was 40 ng/ml with use of a 200 ml lyophilized denatured blood sample.

Pharmacokinetic analysis. Interferon alfa-2a is rapidly and completely absorbed after subcutaneous administration.[22,23] After a peak is reached, the serum levels follow a monoexponential decay. Therefore a one-compartment open model with first-order absorption and first-order elimination was used to describe serum level (C_s) data for both interferon alfa-2a and PEG–interferon alfa-2a. During the initial attempts to describe the data, the fitted values for the absorption rate constant (k_a) and the elimination rate constant (k_e) were indistinguishable. Therefore, L'Hópital's rule was used to solve the differential equation for a one-compartment model. The equation for this model is given below:

$$C_s(t) = [(F \cdot \text{Dose} \cdot k' \cdot t)/(Vd/F)] \cdot e^{-k' \cdot t} \quad (1)$$

in which k′ is the first-order rate constant used to describe the observed serum level time curve, such that $k' = k_a = k_e$, and Vd/F is the volume of distribution, corrected for the bioavailable fraction (F). Data were analyzed with use of the nonlinear least-squares regression program PCNONLIN (version 4.2; Scientific Consulting Inc., Apex, N.C.).

There were insufficient measurable interferon alfa-2a serum level data available from this study to estimate model parameters, so it was necessary to develop the pharmacokinetic model with data from a recently conducted bioequivalence study of single subcutaneous doses of 6.6 MIU interferon alfa-2a in healthy male volunteers (n = 20, data on file, Hoffmann-La Roche, Inc., Nutley, N.J.). Naive pooled data was used to develop the model. This study was selected because of the similarities between subjects, route, and dose. Once the parameter values for interferon alfa-2a were determined, predicted serum level–time profiles were generated and compared to actual data obtained from this study to assess the usefulness of this approach. For PEG–interferon alfa-2a, fits with naive pooled serum level–time data from all dose groups and simultaneous fits of naive pooled serum level–time data from the 9 and 18 MIU dose groups were carried out with use of the model described above. Although the practice of modeling pooled data is not optimal, the primary objective of this exercise was to provide a means of predicting serum levels of interferon alfa-2a and PEG–interferon alfa-2a for input into the pharmacodynamic model.

Pharmacodynamic analysis. MX protein production is characterized by a rapid onset and protracted duration of measurable response after the administration of interferon alfa-2a.[11,12] The exact mechanism of induction is not completely defined. However, the mechanism of MX induction can be simplified in a manner analogous to work presented by Jusko and Ko[24] on the pharmacodynamics of the β_2-adrenergic agonists. Because this analysis involved the assessment of intracellular induction of MX protein in peripheral blood monocytes, there was no need to account for diffusion related phenomena. Therefore, MX protein induction could be fitted with use of the basic physiologic indirect response model described by Dayneka et al.[25] This model is schematically represented in Fig. 1. The differential equation used to describe this model is shown below:

$$\frac{dMXA}{dt} = k_{in} \cdot \left[1 + \frac{E_{max} \cdot C_s}{EC_{50} + C_s}\right] - k_{out} \cdot MXA \quad (2)$$

in which k_{in} is the zero-order rate constant for the production of MX protein, E_{max} is the maximum

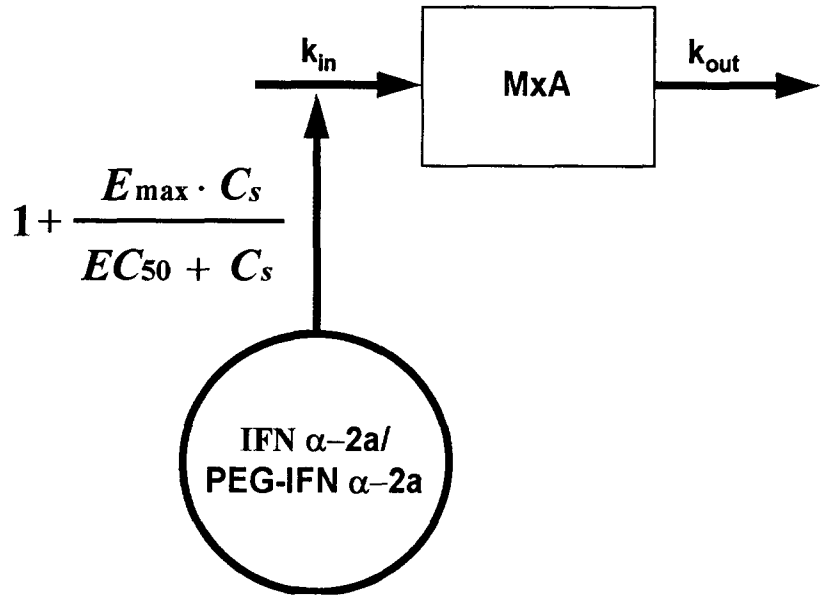

Fig. 1. Schematic representation of pharmacodynamic model for interferon (IFN) alfa-2a and polyethylene glycol (PEG)–interferon alfa-2a effect on MX protein. k_{in}, Zero-order rate constant for production of MX protein; k_{out}, first-order rate constant for decline of MX protein; E_{max}, maximum attainable stimulation of k_{in} by interferon alfa-2a and PEG–interferon alfa-2a; EC_{50}, level of interferon alfa-2a and PEG–interferon alfa-2a that produces 50% of maximum attainable stimulation; C_s, interferon alfa-2a and PEG–interferon alfa-2a level.

attainable stimulation of k_{in} by interferon alfa-2a or PEG–interferon alfa-2a, EC_{50} is the serum level of interferon alfa-2a or PEG–interferon alfa-2a that produces 50% of the maximum attainable stimulation of k_{in}, and k_{out} is the first-order rate constant for decline of MX protein. Data were analyzed with use of the nonlinear least-squares regression program PCNONLIN (version 4.2).

Serum level data for interferon alfa-2a were simulated with use of the pharmacokinetic parameters obtained from the bioequivalence study data. Serum level data for PEG–interferon alfa-2a were simulated with the pharmacokinetic parameters obtained from a simultaneous fit of the 9 and 18 MIU dose groups because the majority of available serum level observations were from these groups. Initial parameter estimates were obtained with use of the method described by Dayneka et al.[25] Model performance was judged by visual assessment of diagnostic plots and by calculation of mean prediction error and absolute mean prediction error. No weighting scheme was used to fit the model. Simultaneous fits of naive pooled MX protein time data from all dose groups (3, 6, 9, and 18 MIU) for interferon alfa-2a and PEG–interferon alfa-2a were performed to obtain pharmacodynamic parameter estimates for both interferon alfa-2a and PEG–interferon alfa-2a, respectively. A simultaneous fit with use of both interferon alfa-2a and PEG–interferon alfa-2a data was also performed. In this second procedure, a common k_{in}, k_{out}, and EC_{50} were estimated for both drugs. A separate E_{max} was estimated for interferon alfa-2a and PEG–interferon alfa-2a as a result of findings from previous fits of the interferon alfa-2a and PEG–interferon alfa-2a groups.

RESULTS

Pharmacokinetics. Plots of observed and predicted serum levels of interferon alfa-2a versus time are shown in Fig. 2. Fig. 2, *A,* shows the ability of the model to describe the data from the 6.6 MIU bioavailability study; Fig. 2, *B,* shows the predicted serum level–time profile for an 18 MIU dose of interferon alfa-2a superimposed on the observed serum levels obtained in this study. Although some lack of fit is noted, the pharmacokinetic model provides a reasonable estimate of exposure to interferon alfa-2a for use in the pharmacokinetic-

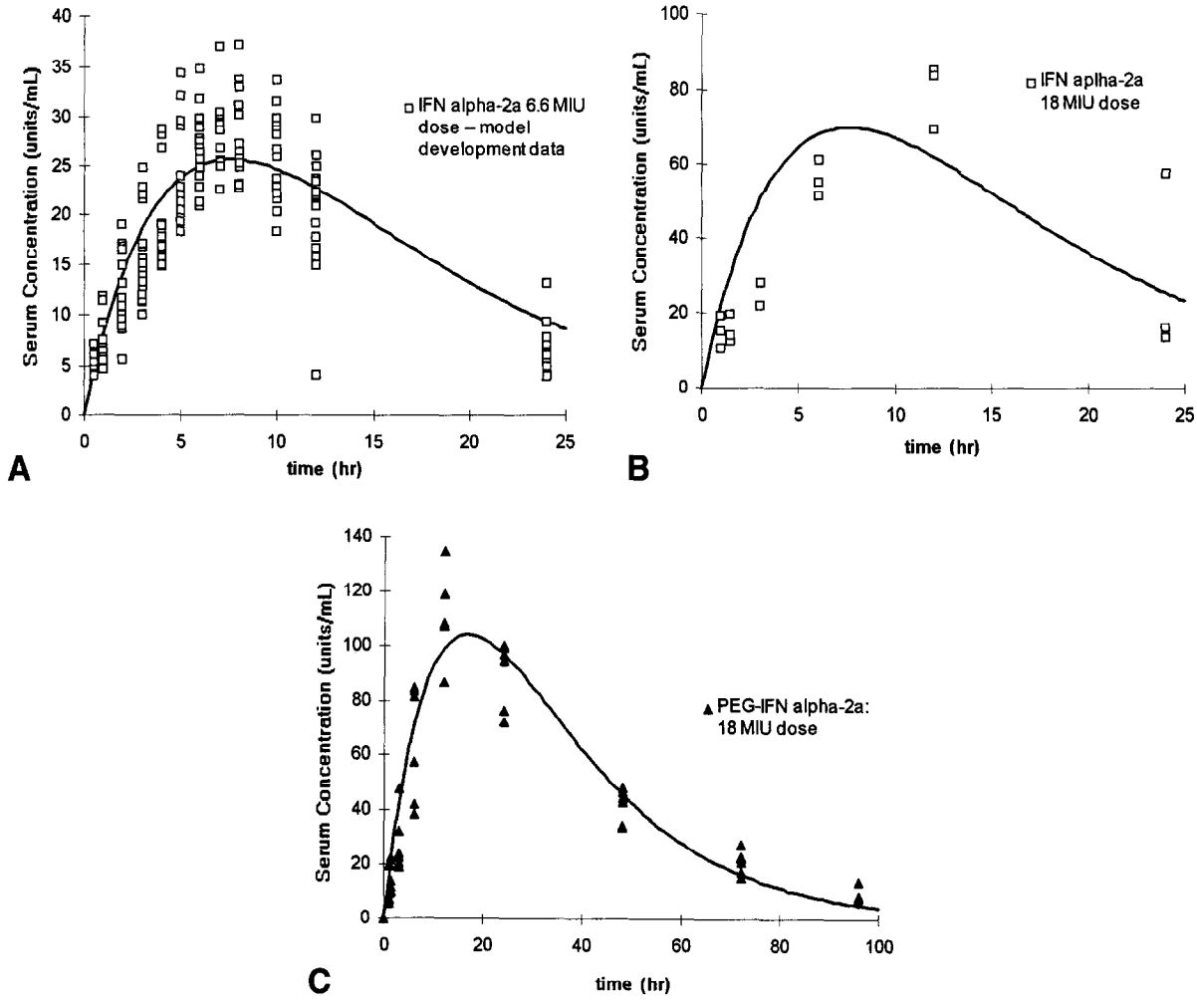

Fig. 2. Individual and model predicted interferon alfa-2a serum level–time profiles after single subcutaneous doses of 6.6 MIU interferon alfa-2a (**A;** pharmacokinetic model development data, $n = 20$) and 18 MIU interferon alfa-2a (**B;** present study data, $n = 3$). Model parameters for interferon alfa-2a were derived by least-squares curve fitting of the data show in panel **A** (recently conducted bioequivalence study, data on file, Hoffmann-La Roche, Inc.) by equation 1. *Symbols* represent experimental data; *solid lines* represent pharmacokinetic model predictions. **C,** Individual and model predicted PEG–interferon alfa-2a serum level–time data after a single subcutaneous dose of 18 MIU PEG–interferon alfa-2a. *Solid line* represents pharmacokinetic model prediction based on least-squares curve fitting by equation 1; *symbols* represent experimental data.

pharmacodynamic model. A plot of observed and model predicted serum level–time data for the 18 MIU dose groups of PEG–interferon alfa-2a is shown in Fig. 2, *C*.

The estimated pharmacokinetic model parameters are presented in Table I. The apparent half-life of interferon alfa-2a was estimated to be 5.3 hours. The pharmacokinetic parameters for interferon alfa-2a are in good agreement with previously published values.[22,23] The pharmacokinetic linearity of interferon alfa-2a in healthy volunteers within this range of doses has been previously established.[22] For PEG–interferon alfa-2a, the apparent half-life was estimated to be 11.9 hours. The consistency of the pharmacokinetic model parameter estimates across all dose groups of PEG–interferon alfa-2a, as

Table I. Model-dependent pharmacokinetic parameters for IFN alfa-2a and PEG–IFN alfa-2a

Drug	*Dose group (MIU)*	*n*	*Parameter (SE)**	
			k' (hr^{-1})	*Vd/F (L)*
IFN alfa-2a†	6.6	20	0.131 (0.006)	94.8 (3.1)
PEG-IFN alfa-2a	3	12	0.076 (0.006)	35.2 (3.8)
	6	6	0.077 (0.010)	35.4 (9.2)
	9	6	0.064 (0.003)	55.0 (2.5)
	18	6	0.058 (0.002)	65.8 (1.7)
	Mean (SD)‡	30	0.069 (0.009)	47.9 (15.1)
	9 and 18 simultaneously	12	0.060 (0.002)	63.7 (1.8)

IFN, Interferon; PEG, polyethylene glycol; MIU, million international units.
*k', First-order rate constant, where k' = absorption rate constant (k_a) = elimination rate constant (k_e); Vd/F, volume of distribution corrected for the bioavailable fraction *(F)*.
†Model parameters derived from serum level–time data taken from recently conducted bioequivalence study of single subcutaneous 6.6 MIU dose of IFN alfa-2a (data on file, Hoffmann-La Roche).
‡Overall mean (SD) parameter estimate from separate, naive pooled fits of 3, 6, 9, and 18 MIU PEG-IFN alfa-2a dose groups.

demonstrated by the low standard deviation, is indicative of the linear kinetics of this compound.

Pharmacodynamics. Measurable increases in MX protein concentration occurred within 1 to 3 hours after dosing of both drugs. Peak response occurred within 24 to 48 hours. MX protein did not return to baseline values within the study period for the 9 and 18 MIU PEG–interferon alfa-2a dose groups. Plots of measured and predicted MX concentrations are shown for interferon alfa-2a and for PEG–interferon alfa-2a in Fig. 3 and Fig. 4, respectively. There is good agreement between observed and predicted MX concentrations, indicating that this model is able to describe the induction of MX protein by both interferon alfa-2a and PEG–interferon alfa-2a. The estimated pharmacodynamic parameters are given in Table II. The relatively low values of the standard error reflect the fact that the parameters are well defined for this model.

Simulated profiles of MX protein induction after therapeutic dose regimens for interferon alfa-2a were generated and compared with the simulated MX responses for various proposed dose regimens for PEG–interferon alfa-2a. Simulations assumed a dose schedule that is frequently used by patients (e.g., Monday, Wednesday, and Friday for a three-times-a-week dose regimen). The reference regimen was a 6 MIU dose of interferon alfa-2a administered three times weekly. The reference dose regimen was chosen as the comparator because it is the current recommended dose regimen for the treatment of hepatitis C and has been shown to have efficacy. For PEG–interferon alfa-2a, simulations were made for a 6 MIU dose given one, two, or three times a week.

To assess the theoretical efficacy of the various test regimens, it was assumed that some minimum concentration of MX protein (MX_{min}) was necessary for efficacy. It was also assumed that the results of the pharmacokinetic-pharmacodynamic analysis of these single doses could be extrapolated to the multiple-dose situation. Fig. 5 shows the simulated MX profiles for the test regimens compared with the reference regimen. The MX_{min} value is delineated on these plots by the broken line. It can be seen in Fig. 5 that both the two-times-a-week and the three-times-a-week PEG–interferon alfa-2a dose regimens are comparable to a three-times-a-week reference regimen of interferon alfa-2a in terms of MX_{min}. The once-weekly regimen for PEG–interferon alfa-2a is clearly inferior. This simulation exercise, together with findings from later phase II trials that substantiate the results from this exercise, showed the utility of the use of MX protein as a pharmacodynamic probe to compare the in vivo activity of interferon alfa-2a and PEG–interferon alfa-2a.

DISCUSSION

Pharmacokinetics. The covalent attachment of PEG moieties is thought to protect therapeutic proteins from breakdown by acting as a physical

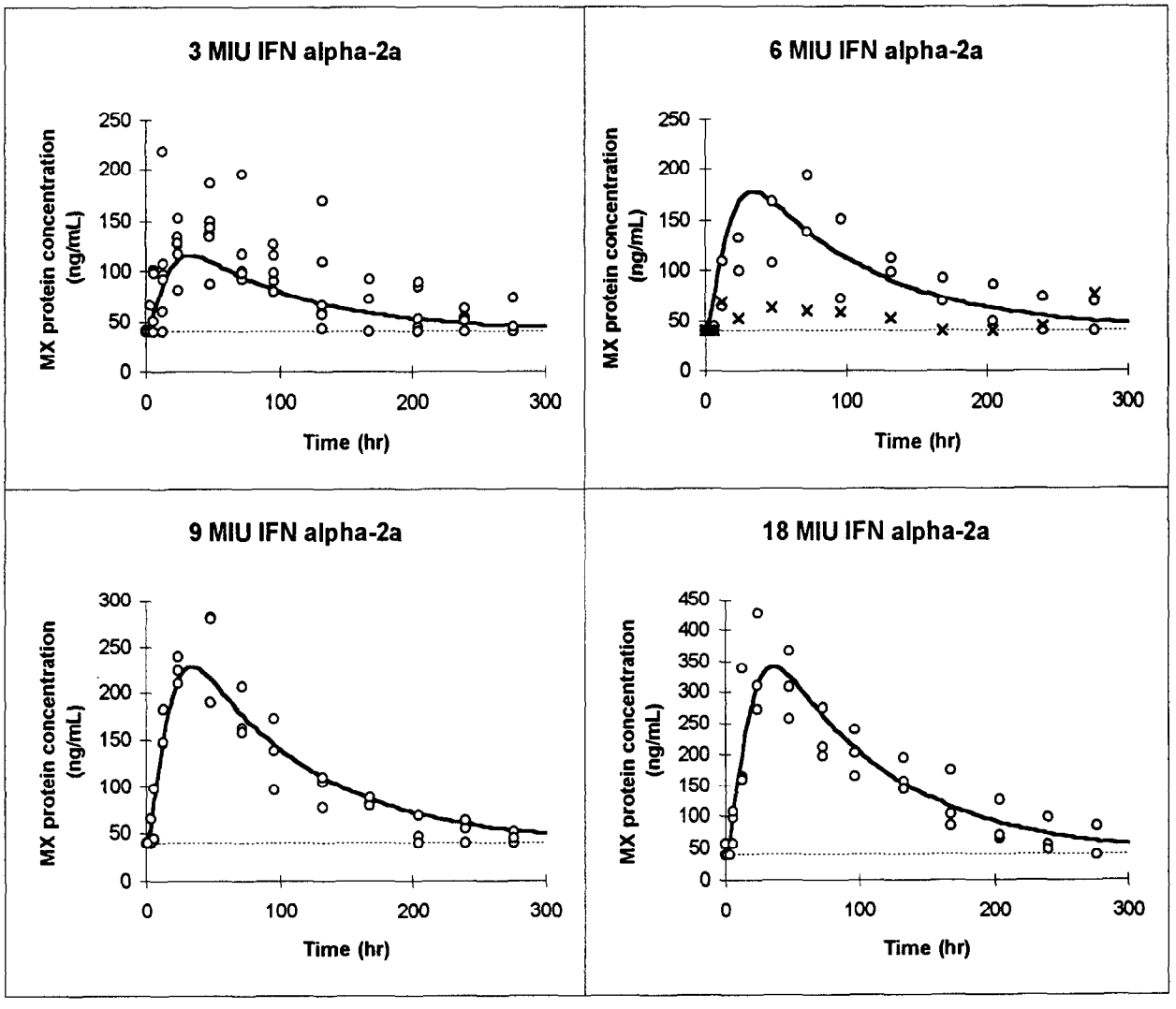

Fig. 3. Individual and model predicted MX protein serum concentration–time data after single subcutaneous doses of 3 (**top left panel;** n = 6), 6 (**top right panel;** n = 3), 9 (**bottom left panel;** n = 3), and 18 (**bottom right panel;** n = 3) MIU interferon alfa-2a. *Solid lines* represent pharmacodynamic model predictions based on least-squares curve fitting by equation 2; *symbols* represent experimental data. One subject in the 6 MIU dose group *(x symbol)* was excluded from the pharmacokinetic-pharmacodynamic analysis.

barrier to proteolytic digestion and by preventing recognition by the immune system.[14-16,18] These actions have been shown to extend the half-life of the protein. It was therefore expected that modification of interferon alfa-2a would extend the half-life of the PEG-modified interferon alfa-2a and would allow for a less frequent dosing interval, thus offering a clinical advantage over currently available interferon alfa-2a formulations.

Serum levels of PEG–interferon alfa-2a declined monoexponentially, with an apparent half-life of 12 hours. This represents a greater than twofold increase in the half-life caused by chemical modification and is consistent with findings noted with other PEG-modified proteins.[15] However, this finding needs to be substantiated by characterizing the intravenous pharmacokinetic behavior of PEG–interferon alfa-2a.

Pharmacodynamics. Modification of a protein by the covalent attachment of PEG has not been

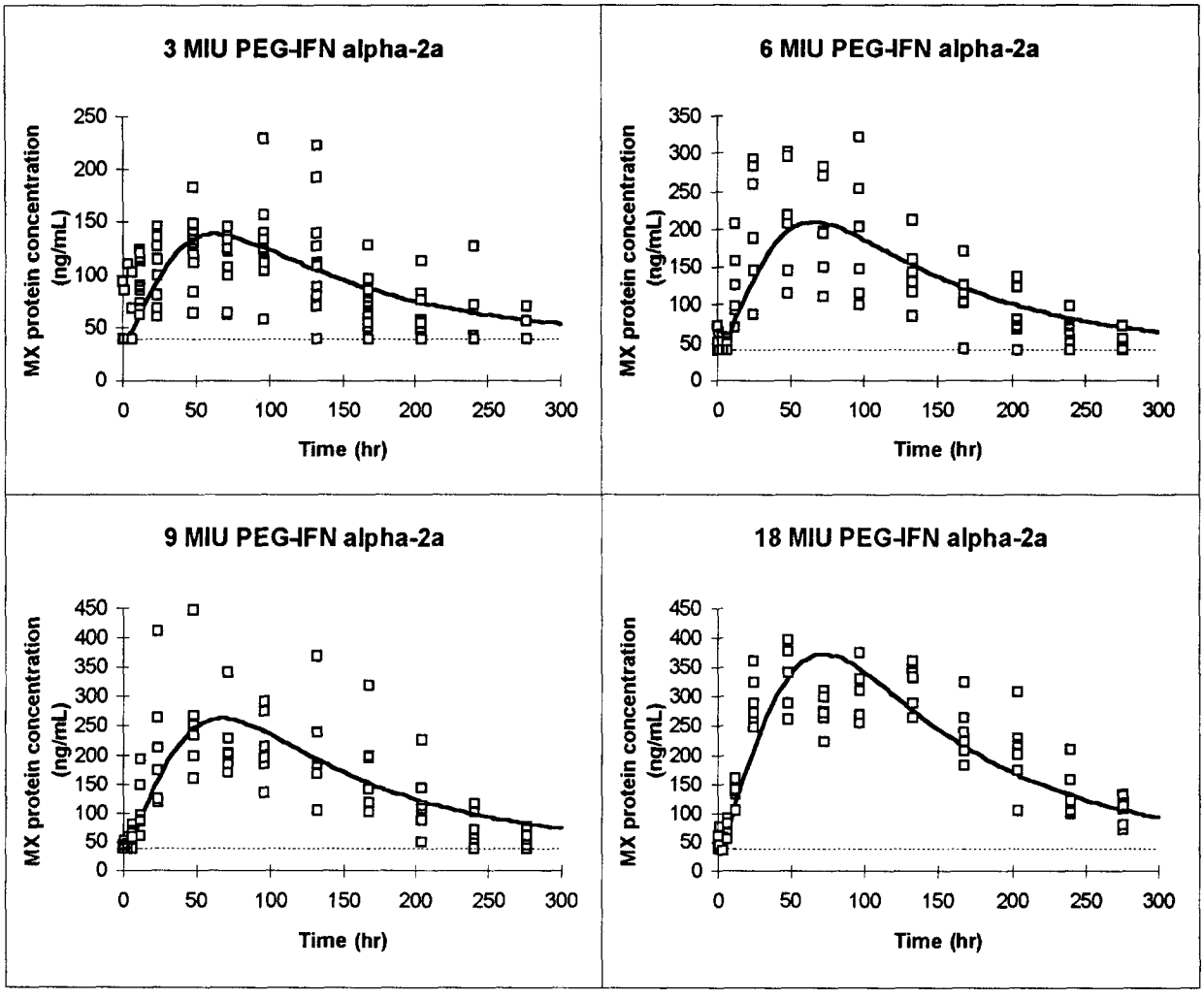

Fig. 4. Individual and model predicted MX protein serum concentration–time data after single subcutaneous doses of 3 (**top left panel;** $n = 11$), 6 (**top right panel;** $n = 6$), 9 (**bottom left panel;** $n = 6$), and 18 (**bottom right panel;** $n = 6$) MIU PEG–interferon alfa-2a. *Solid lines* represent pharmacodynamic model predictions based on least-squares curve fitting by equation 2; *symbols* represent experimental data. MX protein samples were lost for one subject in the 3 MIU dose group.

shown to affect the in vivo mechanism of action of therapeutic proteins.[15,16,26] The pharmacodynamic parameters obtained from the fits for both interferon alfa-2a and PEG–interferon alfa-2a are similar, except for the E_{max} values. However, it should be understood that serum levels of interferon alfa-2a and PEG–interferon alfa-2a are reported here in units of activity (units per milliliter) rather than in mass units, such as nanograms per milliliter. The measured specific activity of PEG–interferon alfa-2a (15 mg/MIU) was one-third that of interferon alfa-2a (5 mg/MIU). Adjusting the serum level–time data to reflect mass units of protein and refitting the pharmacodynamic model would similarly increase the EC_{50} values for PEG–interferon alfa-2a.

There were notable differences between the E_{max} values determined for interferon alfa-2a and PEG–interferon alfa-2a. The differences in the E_{max} values may be partly ascribed to structural changes in the protein by the attachment of a large molecule. Stearic interactions of the high

Table II. Pharmacodynamic parameters for MX protein induction by IFN alfa-2a and PEG-IFN alfa-2a

Drug	Parameter (SE)*				
	k_{in} (hr^{-1})	k_{out} (hr^{-1})	E_{max} IFN alfa-2a	E_{max} PEG-IFN alfa-2a	EC_{50} (units/ml)
IFN alfa-2a†	0.44	0.011 (0.0008)	67.2 (14.3)	—	63.5 (18.8)
PEG-IFN alfa-2a†	0.36	0.009 (0.0008)	—	39.2 (8.2)	61.1 (19.3)
Combined‡	0.40	0.010 (0.0006)	68.3 (10.9)	36.4 (5.1)	58.3 (12.6)

*k_{in}, Zero-order rate constant for production of MX protein; k_{out}, first-order rate constant for decline of MX protein; E_{max}, maximum attainable stimulation of k_{in} by IFN alfa-2a and PEG-IFN alfa-2a; EC_{50}, level of IFN alfa-2a/PEG-IFN alfa-2a that produces 50% of maximum attainable stimulation.
†Simultaneous fit of 3, 6, 9, and 18 MIU dose groups.
‡Combined fit of both IFN alfa-2a and PEG-IFN alfa-2a 3, 6, 9, and 18 MIU dose groups.

molecular weight PEG may interfere with the binding of interferon alfa-2a to cell surface receptors. Diminished activity has been reported with other PEG-modified proteins, although these proteins were larger proteins with small diffusible substrates.[14,26]

Comparison of pharmacodynamic response as a means to evaluate relative activity and refine therapeutic dose regimens has been done previously.[12] In addition, a model describing the indirect stimulation of neopterin production as a means of assessing macrophage activation after a single subcutaneous dose of interferon alfa-2a has also been described (data on file, Hoffmann-La Roche). MX protein induction is linear within the range of doses tested, and the response is maintained during repeated dosing in patients.[10,27] Despite the fact that this was a single-dose study conducted in healthy volunteers, it was reasonable to extend this work into simulated multiple-dose regimens. For purposes of providing guidance for dose regimen selection in patients, it was necessary to assume that the MX response in patients with hepatitis C is similar to that observed in healthy volunteers. Dose-proportional sustained increases of peripheral blood monocyte levels of MX protein have been measured in patients with cancer during treatment with interferon alfa-2b,[27] and the temporal response of MX protein to interferon alfa-2b in these patients was very similar to the response observed in the present study. Assessment of the calculated MX protein concentrations for both interferon alfa-2a and for PEG–interferon alfa-2a for the 6 MIU dose regimens indicates that the pharmacodynamic response obtained by interferon alfa-2a given three times weekly can not be accomplished by once weekly administration of PEG–interferon alfa-2a. The predicted MX protein concentration–time profiles indicate that at least twice weekly administration of PEG–interferon alfa-2a would be necessary to equal the pharmacodynamic response obtained with three-times-a-week dosing of interferon alfa-2a. Comparison of simulated MX profiles in Fig. 5 is also supportive of the fact that a once-a-week 6 MIU dose regimen for PEG–interferon alfa-2a would not be appropriate. MX_{min} values fall too low, possibly allowing breakthrough viral replication, with a once-weekly PEG–interferon alfa-2a treatment regimen. Two- or three-times-a-week 6 MIU PEG–interferon alfa-2a regimens have sufficient MX_{min} values. This finding of dose frequency was later substantiated in two phase II trials in patients with hepatitis C (data on file, Hoffmann-La Roche, Inc.), in which the once-weekly PEG–interferon alfa-2a regimen proved to be inferior to the reference regimen of interferon alfa-2a given three times a week.

There are several potential shortcomings to pooling pharmacokinetic-pharmacodynamic data for modeling as was done in this analysis. First, bias can be introduced if the measured response varies markedly among individuals, as would be the case if certain individuals were substantially more or less "sensitive" than "normal" individuals to the effects of a drug. A pharmacokinetic-pharmacodynamic model developed from pooled data in such a situation may not describe the dynamics of either type of individual correctly. Second, pooling data provides no means to identify and characterize potential sources of interindividual variability. This study attempted to minimize the potential for bias by controlling for likely potential sources of variability in the study population such as subject weight, age, and gender. Also, one subject in the 6 MIU interferon

alfa-2a dose group was deleted from the pharmacokinetic-pharmacodynamic analysis because the pharmacodynamic response in this subject deviated enough to suggest that either a study error occurred or that this subject was truly different from the others with respect to the MX protein response to interferon alfa-2a administration. Despite the disadvantages inherent in pooling data, this approach allowed useful information that was critical to drug development to be provided in a timely fashion.

MX protein induction has not been directly correlated with clinical response in hepatitis C. However, there have been attempts to investigate the relationship between MX protein induction and clinical response in other diseases during treatment with α-interferons.[27] To validate the results of this study it would be necessary to run a clinical trial specifically designed to correlate MX protein levels with clinical response in patients with hepatitis C. To date, more than 50 interferon regulated proteins have been described in the literature, and it is unlikely that any one interferon-regulated protein will correlate to all interferon induced activities. However, MX protein exerts a myriad of effects on cellular processes, has intrinsic antiviral and antitumor activity, and has a linear sustained response in patients. Furthermore, the production of MX protein in response to interferon alfa-2a is ubiquitous in all tissues. MX proteins may therefore be uniquely suited to show the in vivo activities of interferons in some therapeutic areas.

In conclusion, we have presented an indirect physiologic response model to characterize the biological activity of interferon alfa-2a and PEG–interferon alfa-2a as measured by the induction of MX protein. Comparison of the pharmacokinetics of the two compounds indicated that covalent attachment of a PEG moiety increased the circulating half-life of interferon alfa-2a by more than twofold. However, pharmacodynamic analysis was a more useful indication of the potential therapeutic utility of the modified interferon alfa-2a. Simulations of projected dose regimens showed that this compound had limited therapeutic benefit over conventional interferon alfa-2a, and these results were consistent with findings in phase II trials. This work substantiates the usefulness of pharmacodynamic modeling as a tool for the development of dose recommendations and for the selection of drug candidates in the drug development process.

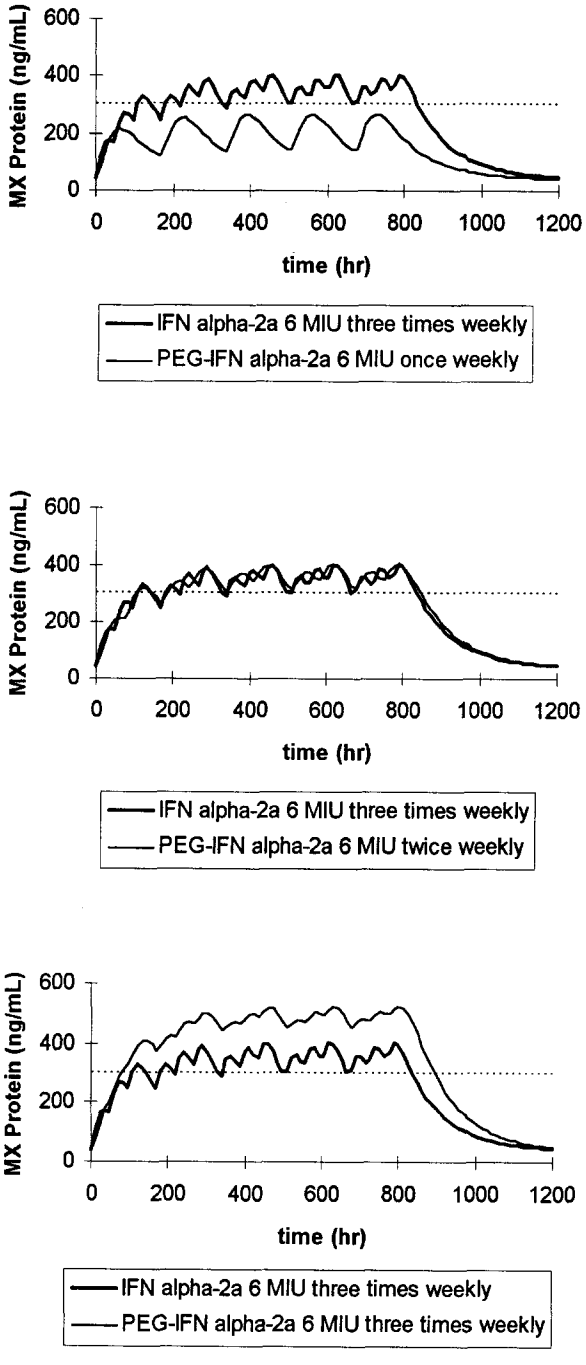

Fig. 5. Simulated MX protein concentration–time profiles after once-weekly (**top panel**), twice-weekly (**middle panel**), and thrice-weekly (**bottom panel**) subcutaneous administration of 6 MIU PEG–interferon alfa-2a *(light solid lines). Dark solid lines* represent reference dosing regimen of 6 MIU interferon alfa-2a administered thrice weekly; *broken lines* represent trough MX protein concentration at steady state.

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