A Pharmacokinetic/Pharmacodynamic Model for Recombinant Human Growth Hormone Effects on Induction of Insulin-Like Growth Factor I in Monkeys\(^1\)

YU-NIEN SUN, HYE JUNG LEE, RICHARD R. ALMON, and WILLIAM J. JUSKO

Department of Pharmaceutical Sciences, School of Pharmacy (Y.-N.S., R.R.A., W.J.J.), and Department of Biological Sciences (R.R.A.), State University of New York at Buffalo, Buffalo, New York; and Alkermes, Inc., Cambridge, Massachusetts (H.J.L.)

Received for publication November 10, 1998. This work was supported by Alkermes, Inc. and by Grants GM 24211 and AG 10629 from the National Institute of General Medical Sciences and the National Institute on Aging, National Institutes of Health.

\(^1\) This work was supported by Alkermes, Inc. and by Grants GM 24211 and AG 10629 from the National Institute of General Medical Sciences and the National Institute on Aging, National Institutes of Health.

ABSTRACT

The pharmacokinetics of recombinant human growth hormone (rhGH) and its effects on the induction of insulin-like growth factor I (IGF-I) were studied in juvenile rhesus monkeys. Disposition profiles of rhGH from two short-term i.v. infusion studies were described by a two-compartment model yielding a clearance of 16.1 ml/min and a terminal halflife of 2.0 h. Four rhGH treatment groups were included in this study: group A, ProLease rhGH (24 mg), a sustained-release microsphere formulation; group B, a single s.c. injection plus an implanted osmotic pump (24.4 mg); group C, a single s.c. injection (25.9 mg); group D, daily 0.86-mg s.c. injection for 28 days. Their rhGH input profiles were analyzed by a numerical deconvolution method. ProLease and osmotic pump provided zero-order inputs of rhGH and maintained the serum rhGH concentrations around 9 to 13 ng/ml for 16 (group A) and 30 days (group B). For s.c. injections, rhGH underwent first-order absorption. An indirect response model was applied based on use of a Hill function for stimulation of IGF-I production. Parameter values obtained included \(S_{\text{max}} = 2.2\), \(SC_{50} = 6.5\) ng/ml, and \(\gamma\) (slope coefficient) = 6.8, which were applicable to all treatments. The area under effect curve showed group B to be most effective for IGF-I induction, whereas group A produced the highest peak level in 16 days. Group C had the lowest induction among the four groups, despite being given the highest dose. Group D had modest IGF-I induction, but the pulsatile rhGH input is less effective than continuous input provided by ProLease. Our pharmacokinetic/pharmacodynamic model demonstrates that ProLease and osmotic pump delivery were best able to maintain rhGH level above the s.c.50 value, which provided more effective IGF-I induction compared with the single or daily subcutaneous injections in solution.

ABBREVIATIONS: rhGH, recombinant human growth hormone; IGF-I, insulin-like growth factor I; PK/PD, pharmacokinetics/pharmacodynamics; AUC, area under the curve; AUEC, area under the effect curve; CL, clearance; \(V_{\text{c}}\), volume of distribution in the central compartment; \(V_{\text{sc}}\), volume of distribution at the steady state; \(k_{\text{a}}\), first-order absorption rate constant; \(k_{12}\) and \(k_{21}\), distribution rate constants; \(\lambda_1\) and \(\lambda_2\), slope coefficients; \(k_{\text{el}}\), elimination rate constant; \(F\), bioavailability; s.c., subcutaneous; MRT, mean residence time; \(K_{\text{el}}\), IGF-I formation rate; \(K_{\text{out}}\), IGF-I elimination rate; \(S_{\text{max}}\), maximum stimulation of IGF-I formation rate; \(SC_{50}\), rhGH concentration producing 50% of the maximum effect; \(\gamma\), slope coefficient for the Hill function; HX, hypophysectomized; GHBP, growth hormone-binding protein.
major role in the regulation of IGF-I, IGFBP-3, and acid-labile subunit (Kupfer et al., 1993).

The effectiveness from continuous infusion compared with pulsatile GH injections remains a major issue in therapy. In hypophysectomized (HX) rats, the increase of IGF-I in rat skeletal tissues is more effective by pulsatile i.v. GH infusion than by continuous infusion (Isgaard et al., 1988). However, rhGH continuous infusion induced serum IGFI-I and GH binding protein (GHBP) in a dose-dependent manner, whereas single s.c. daily injections were ineffective in HX rats (Clark et al., 1995). The same study showed that twice-daily s.c. injections of rhGH were slightly more effective than continuous infusion for body weight gains, whereas single-daily injections were the least effective. In GH-deficient human adults, serum IGF-I induction was greater by continuous infusion compared with the same daily dose as eight i.v. injections every 3.5 h (Laursen et al., 1994). These results suggest that a sustained-release formulation of rhGH may achieve its pharmacological effects as the conventional daily injections by maintaining the serum hormone concentrations above a certain level over time.

In this report, a sustained-release formulation ProLease rhGH was studied in juvenile rhesus monkeys. ProLease is composed of biodegradable microspheres containing the desired bioactive molecule incorporated into a matrix of poly(DL-lactide-co-glycolide). In vitro and in vivo characteristics of ProLease rhGH have been reported elsewhere (Johnson et al., 1996). To test the effectiveness of rhGH delivered by ProLease, four different treatment groups of monkeys were given similar total rhGH doses (24.0 to 25.9 mg). They included rhGH in ProLease by s.c. injection, single s.c. injection plus osmotic pump delivery of rhGH (to mimic the initial release and continuous delivery of rhGH by ProLease), single s.c. injection of rhGH, and daily s.c. injections of rhGH (Johnson et al., 1996; Lee et al., 1997). Pharmacokinetic/pharmacodynamic (PK/PD) modeling was enacted to describe the pharmacokinetic profiles of rhGH for these four treatment groups and the relationships between rhGH serum concentrations and IGF-I induction. The disposition of rhGH in monkeys by two short-term i.v. infusions of the hormone yielded its primary pharmacokinetic parameters. Numerical deconvolution method (Cutler, 1978; Venge-Pedersen, 1988) was then applied to assess the input profiles of the four treatments using i.v. data as the disposition function. These profiles were used to identify the input functions for the pharmacokinetic analysis. For IGF-I induction, an indirect response model (Dayneca et al., 1993) was applied. The SCSo rhGH potency factor for IGF-I induction was estimated.

Materials and Methods

Study Design

For the rhGH disposition study, four juvenile rhesus monkeys (body weight, b.wt. = 2.3 ± 0.05 kg) were given two different doses of rhGH, 0.10 mg/kg and 1.42 mg/kg, by 5-min short-term infusions. These studies were separated by a washout period of 7 days. Serum rhGH concentrations were determined at 16 times up to 12.08 h after each dose. Mean values at each time point from these four monkeys were used in the pharmacokinetic analysis.

**Group A (ProLease rhGH).** One hundred sixty micrograms of microspheres (24.0 mg of rhGH) were given by s.c. injection to each of the four monkeys (b.wt. = 3.3 ± 0.2 kg) on day 0. Serum rhGH concentrations were determined at nine times up to 12 h of the first day, and on 24 days up to day 75. Serum IGF-I concentrations were determined at six times up to 12 h of the first day, and on 23 days up to day 60.

**Group B (Single s.c. Plus Pump).** A single s.c. injection of 3.6 mg of rhGH was given, and an osmotic pump containing 20.8 mg of rhGH was surgically implanted in each of four monkeys (b.wt. = 3.2 ± 0.2 kg) on day 0. Pumps were removed on day 30. Serum rhGH and IGF-I concentrations were determined at essentially the same times as in group A.

**Group C (Single rhGH s.c.).** A single s.c. injection of 25.9 mg of rhGH was given to each of four monkeys (b.wt. = 3.3 ± 0.2 kg) daily from day 0 to day 27 (total of 28 days). Serum rhGH concentrations were determined at nine times up to 12 h of the first day; predose and 2 h postdose on days 1, 2, 3, 5, 7, 13, and 19; predose and seven times postdose on day 27 and days 28, 34, 38, 40, 47, 49, 52, 55, and 74. Serum IGF-I concentrations were determined at seven times up to 12 h of the first day, 2 h postdose on 8 days up to day 27, and then on 6 days up to day 55.

**Group D (Daily rhGH s.c.).** A single s.c. injection of 0.86 mg of rhGH was given to each of four monkeys (b.wt. = 3.0 ± 0.2 kg) daily from day 0 to day 75 (total of 28 days). Serum rhGH concentrations were determined at six times up to 12 h of the first day; predose and 2 h postdose on days 1, 2, 3, 5, 7, 13, 19; predose and seven times postdose on day 27 and days 28, 34, 38, 40, 47, 49, 52, 55, and 74. Serum IGF-I concentrations were determined at seven times up to 12 h of the first day, 2 h postdose on 8 days up to day 27, and then on 6 days up to day 55.

Serum rhGH concentrations were determined by immunoradiometric assay (Radim Group, Rome, Italy). IGF-I was separated from the binding proteins by acid/ethanol extraction, and the serum concentrations were determined by radioimmunoassay (Diagnostic System Laboratories, Inc., Webster, TX). Details of the methodology are reported elsewhere (Johnson et al., 1996; Lee et al., 1997).

Pharmacokinetic Analysis

**Disposition of rhGH.** The biexponential disposition of rhGH serum concentrations from two short-term infusion studies can be described by a two-compartment model (shown in Fig. 1) as follows:

\[
\frac{dA_p}{dt} = -\left(\frac{CL}{V_c}\right) \cdot A_p - k_{12} \cdot A_p + k_{21} \cdot A_1 + R_i(0) \quad (1)
\]

\[
\frac{dA_1}{dt} = k_{12} \cdot A_p - k_{21} \cdot A_1 \quad (2)
\]

\[
C_p = \left(\frac{A_p}{V_c}\right) + Base \quad (3)
\]

where \(A_p\) is the amount of rhGH in the central compartment, \(C_p\) is the serum rhGH concentration, \(A_1\) is the amount of rhGH in the peripheral compartment, \(CL\) is clearance, \(V_c\) is the central volume of distribution, \(k_{12}\) and \(k_{21}\) are the distribution rate constants between the central and peripheral compartments, \(R_i(0)\) is the zero-order infusion rate into the central compartment, and \(Base\) is the baseline serum rhGH concentration. Equations 1 to 3 were fitted simultaneously for the two dose levels to estimate \(CL\), \(V_c\), \(k_{12}\), and \(k_{21}\) values by the maximum likelihood method using the ADAPT II program (D’Argenio and Schumitzky, 1997). The estimated parameters were then used as constants in the pharmacokinetic analysis for the four treatment groups.

The elimination rate constant from the central compartment (\(k_{el}\)) is \(CL/V_c\). Considering the 5-min i.v. infusion is practically a bolus dose in a 12-h study, two slope coefficients for the biexponential equation \((C = A \cdot e^{-kt} + B \cdot e^{-kt})\) were calculated from \(k_{el}\), \(k_{12}\), and \(k_{21}\). The terminal phase half-life (\(T_1/2\)) was estimated by 0.693/\(A_2\). Volume of distribution at the steady state (\(V_{u2}\)) was calculated by \(V_c \cdot (1 + k_{12}/k_{21})\). Mean residence time was estimated by \(V_c/CL\). For the distribution clearance of rhGH between compartments, \(CL_d = k_{12} \cdot V_c - k_{21} \cdot V_c\).

Deconvolution Analysis for Input Rates. PCDCON, a deconvolution program for pharmacokinetic applications (Gillespie, 1991),
was applied. The principles for the numerical deconvolution method can be found elsewhere (Cutler, 1978; Veng-Pedersen, 1988). Briefly, when the plasma concentrations versus time profiles for an extravascular administration and an i.v. bolus (dose-normalized as the unit disposition function) of the drug are known, the rate at which drug reaches the system circulation (input rate or absorption rate) for the extravascular administration can be solved numerically by explicit curve-fitting method encoded in the PCDCON program.

The rhGH concentrations from the high-dose short-term infusion were used to obtain the unit disposition function. The input functions representing the absorption profiles of rhGH treatments were then determined. Two profiles are provided. One is “input rate of rhGH versus time” from which the absorption profiles for each treatment can be determined; the other one is “cumulative amount of rhGH versus time” from which the total amount of rhGH absorbed can be assessed. This information was applied to the subsequent pharmacokinetic analysis.

**Pharmacokinetic Analysis of Treatment Groups.** Two-compartment models with different input functions were applied. Depending on the absorption profiles obtained from the numerical deconvolution, zero-order inputs to the central compartment and/or an absorption compartment with a first-order absorption rate constant into the central compartment were assigned (as shown in Fig. 1). The details are as follows.

**Group A (ProLease rhGH).** The results for the analysis of rhGH absorption rates (Fig. 3) showed that the release of rhGH from ProLease has four stages: approximately 4.5 mg/day from day 0 to 24 h, 0.22 mg/day from 24 h to day 17, 0.1 mg/day from day 17 to 60, and no rhGH released afterward. The two-compartment model (Fig. 1) was applied as follows:

\[
\frac{dA_{p,A}}{dt} = \left(\frac{CL}{V_c}\right) \cdot A_{p,A} - k_{12} \cdot A_{p,A} + k_{21} \cdot A_{c,A} + R^*(0)
\]

\[
\frac{dA_{c,A}}{dt} = k_{12} \cdot A_{p,A} - k_{21} \cdot A_{c,A}
\]

\[
C_{p,A} = \left(\frac{A_{p,A}}{V_c}\right) + B_{a2}
\]

where \(R^*(0)\) represents the zero-order input rate, \(A_{p,A}\) is the serum rhGH concentration, and \(B_{a2}\) is the baseline value. Four different stages of inputs were defined as follows: when \(0 < t \leq T_{A1}\), then \(R^*(0) = R_{a1}\); when \(T_{A1} < t \leq T_{A2}\), then \(R^*(0) = R_{A2}\); when \(T_{A2} < t \leq T_{A3}\), then \(R^*(0) = R_{A3}\); when \(t > T_{A3}\), then \(R^*(0) = 0\). Equations 4 to 6 were fitted simultaneously to estimate \(T_{A1}, T_{A2}, T_{A3}, R_{A1}, R_{A2}, R_{A3}\), and \(B_{a2}\) values by the maximum likelihood method using the ADAPT II program (D’Argenio and Schumitzky, 1997).

**Group B (Single s.c. Plus Pump).** The results of the absorption profile (Fig. 4) show that two different rhGH input rates were involved. In the first 12 h, first-order absorption from the single s.c. injection was observed. A zero-order input of about 0.3 mg of rhGH/day delivered by the osmotic pump was seen up to day 30. The equations used were as follows:

\[
\frac{dA_{p,B}}{dt} = -k_{a,B} \cdot A_{p,B}
\]

\[
\frac{dA_{c,B}}{dt} = k_{a,B} \cdot A_{p,B} - k_{12} \cdot A_{p,B} + k_{21} \cdot A_{c,B} + R^*(0)
\]

\[
\frac{dA_{c,B}}{dt} = k_{12} \cdot A_{p,B} - k_{21} \cdot A_{c,B}
\]

where \(k_{a,B}\) is the first-order absorption rate constant, \(C_{p,B}\) is the serum rhGH concentration, and \(B_{a2}\) is its baseline value. The rhGH input rate from the pump was defined as follows: when \(0 < t \leq 30\) days, then \(R^*(t) = R_{a1}\); when \(t > 30\) days, then \(R^*(t) = 0\) (pumps were removed on day 30). The estimates for \(k_{a,B}, A_{c,B}(0)\) (initial condition of eq. 7; total amount of rhGH absorbed from the s.c. injection), \(R_{a1},\) and \(B_{a2}\) were obtained from computer fitting as for group A. The bioavailability of the subcutaneous rhGH injection can be calculated as \(A_{a,B}(0)/\text{injection dose}\).

**Group C (Single rhGH s.c.).** The absorption profile shown in Fig. 5 indicated that first-order absorption was involved in the single rhGH s.c. injection. The equations used were as follows:

\[
\frac{dA_{p,C}}{dt} = -k_{a,C} \cdot A_{p,C}
\]

\[
\frac{dA_{c,C}}{dt} = k_{a,C} \cdot A_{p,C} - \left(\frac{CL}{V_c}\right) \cdot A_{p,C} - k_{12} \cdot A_{p,C} + k_{21} \cdot A_{c,C}
\]

\[
C_{p,C} = \left(\frac{A_{p,C}}{V_c}\right) + B_{a4}
\]

where \(k_{a,C}\) is the first-order absorption rate constant, \(C_{p,C}\) is the serum rhGH concentration, and \(B_{a4}\) is the baseline value. The \(k_{a,C}, A_{c,C}(0)\) (initial condition of eq. 11; total amount of rhGH absorbed from the subcutaneous injection), and \(B_{a4}\) were estimated by computer fitting as for group A. The bioavailability of the subcutaneous rhGH injection can be calculated as \(A_{a,C}(0)/\text{injection dose}\).

**Group D (Daily rhGH s.c.).** The rhGH absorption rate profile for the first injection (Fig. 6) showed that the absorption is a first-order process. Assuming the pharmacokinetics of rhGH are linear and stationary, the serum rhGH concentrations with repeated doses were described as follows:

\[
\frac{dA_{p,D}}{dt} = -k_{a,D} \cdot A_{p,D}
\]

\[
\frac{dA_{c,D}}{dt} = k_{a,D} \cdot F_D \cdot A_{c,D} - \left(\frac{CL}{V_c}\right) \cdot A_{p,D} - k_{12} \cdot A_{p,D} + k_{21} \cdot A_{c,D}
\]

\[
C_{p,D} = \left(\frac{A_{p,D}}{V_c}\right) + B_{a5}
\]

where \(k_{a,D}\) is the first-order rate constant, \(F_D\) is the bioavailability of rhGH for each injection, \(C_{p,D}\) is the serum rhGH concentration, and \(B_{a5}\) is its baseline value. The multiple-dosing schedule was defined in the data file for the ADAPT II program, and \(k_{a,D}, F_D,\) and \(B_{a5}\) were estimated by computer fitting as for group A.

**Area Analysis.** The area under the rhGH serum concentration curve (AUC) from each animal was calculated using the trapezoidal rule. The AUC_total, the mean values of AUC from each treatment group, have been reported elsewhere (Lee et al., 1997). The AUC_set, the area under the concentration curve above the baseline, was calculated by \((\text{AUC}_{\text{total}} - \text{AUC}_{\text{baseline}})\). Baseline values were obtained from computer fittings as described previously.
Pharmacodynamic Analysis: Total IGF-I Induction by rhGH

For the data presented herein, IGF-I concentrations were considered as "total IGF-I" because an acid/ethanol extraction was applied to separate IGF-I from its binding proteins for the assay. We assumed that rhGH can increase the formation rate of protein-bound IGF-I in the serum, whereas the elimination rate of the complex remains unchanged. Indirect response model III (Dayneka et al., 1993) based on stimulation of the formation rate for IGF-I was applied as follows (Fig. 1):

\[
\frac{d IGF_1}{dt} = k_{in} \cdot \left( 1 + \frac{S_{max} \cdot C_{GH}}{SC_{50} + C_{GH}} \right) - k_{out} \cdot IGF_1
\]

where \( IGF_1 \) is the total IGF-I concentration, \( C_{GH} \) is the rhGH serum concentration, which is a forcing function defined by the pharmacokinetic analysis, \( k_{in} \) is the formation rate of IGF-I, \( S_{max} \) is the maximum stimulation of \( k_{in} \), \( SC_{50} \) is the rhGH concentration producing 50% of maximum stimulation of the IGF-I formation rate, \( k_{out} \) is the elimination rate of IGF-I, and \( \gamma \) is the slope coefficient for the Hill function. Assuming that the IGF-I baseline level is maintained by growth hormone baseline concentration \( GH_0 \), and will be restored by a certain time after the dosing, the IGF-I formation rate was defined as follows:

\[
k_{in} = k_{out} \cdot IGF_{10} \left( 1 + \frac{S_{max} \cdot C_{GH,0}}{SC_{50} + C_{GH,0}} \right)
\]

where \( IGF_{10} \) is the initial condition of eq. 20 (IGF-I predose level) and \( C_{GH,0} \) is the baseline rhGH concentration. From data group A (ProLease rhGH) and group B (single s.c. plus pump) were fitted simultaneously. Considering that IGF-I predose levels are different between groups A and B, we assumed that the elimination rate \( k_{out} \) is the same but that formation rates \( k_{in} \) differ between these groups. Pharmacodynamic parameters \( (k_{out}, S_{max}, SC_{50}, \gamma, IGF_{10}) \) were estimated by the maximum likelihood method using the ADAPT II program (D’Argenio and Schumitzky, 1997).

Simulations for the total IGF-I induction by rhGH for group C (single rhGH s.c.) and group D (daily rhGH s.c.) were also performed using the proposed pharmacodynamic model (eqs. 19 and 20) and the estimated parameters. Values of \( IGF_{10} \) were assigned from the predose levels. Total area under the effect curve (AUEC) for total IGF-I inductions were calculated for each group using the trapezoidal rule. The AUEC was area under the effect curve above the IGF-I baseline level, was calculated by AUEC = AUC - AUEC_{max}. The IGF-I baseline levels were from the least-squares estimated values (groups A and B) or assigned predose levels (groups C and D).

Results

Pharmacokinetics

Disposition of rhGH. The serum rhGH concentration profiles from two short-term rhGH infusion studies are shown in Fig. 2. For the high dose, the rhGH concentrations decline biexponentially with a fairly steep distribution phase followed by a shallow elimination phase. For the low dose, there is a steep disposition slope parallel to that of the high dose. However, the second phase is not very obvious. Both profiles have a baseline rhGH of about 3 ng/ml. The estimated parameters from the computer fitting (eqs. 1–3) are listed in Table 1. Our \( CL \), \( V_e \), and \( V_{ss} \) values for rhGH in monkeys are very close to those of Mordenti et al. (1991). The distribution clearance \( (CL_e = 0.35 \text{ ml/min}) \) is much lower than the elimination clearance \( (CL = 16.1 \text{ ml/min}) \), indicating that the steep distribution phase is due to rapid elimination rather than distribution from plasma.

Four rhGH Treatments

ProLease rhGH Dose.

The rhGH concentration versus time and the rhGH absorption profiles are shown in Fig. 3. ProLease delivered rhGH by three different rates and stopped around 60 days postdosing. The cumulative amount of rhGH absorbed at day 60 is about 12 mg, indicating bioavailability of approximately 50%. That is close to results from AUC analysis where the overall bioavailability is 0.47 and the net bioavailability is 0.51 (Table 3). The parameters estimated by computer fitting (eqs. 4–6) are listed in Table 2. During days 0 to 1.3, rhGH was released at a high rate of 4.077 mg/day. During days 1.3 to 16, a moderate rhGH release rate of 0.184 mg/day kept the serum rhGH concentration at a steady state around 9.2 ng/ml. Finally, during days 16 to 62, a low rhGH release rate of 0.063 mg/day maintained the serum rhGH concentration at a steady state about 4.0 ng/ml. After day 62, the rhGH concentration dropped to its baseline value (estimated as 1.3 ng/ml). These results were approximated by the model based on the assumption that rhGH was absorbed by three different constant rates over three different periods. By calculating the sum of (input rate × input duration) for these three zero-order inputs, the total amount of rhGH absorbed is about 10.9 mg (bioavailability = 10.9 mg/24 mg = 0.45). It is consistent with the results from the AUC and deconvolution analyses.
Single s.c. Dose Plus Pump. The rhGH concentration versus time and the rhGH absorption profiles are shown in Fig. 4. The rhGH s.c. injection on day 0 had a monoeponential decline, indicating that first-order absorption was involved. Subsequently, the implanted pump delivered rhGH at a constant rate until the pump was surgically removed (day 30). The parameters estimated by computer fitting (eqs. 7–10) are listed in Table 2. The first-order absorption rate constant for the s.c. injection is 7.75 days^{-1}. The estimated release rate from the pump is 0.295 mg/day, which maintained the serum rhGH concentration at a steady state of about 13.7 ng/ml for 30 days. After that, the rhGH concentration declined to its baseline value (estimated as 0.97 ng/ml). The bioavailability of the single s.c. dose can be estimated by $A_{a,B}$/Injection dose = 2.433/3.6 = 0.68. The overall bioavailability of single s.c. plus pump rhGH administration estimated by the AUC method is 0.50, and the net bioavailability is 0.49 (Table 3).

Single rhGH s.c. Dose. The rhGH concentration versus time and the rhGH absorption profiles are shown in Fig. 5. The absorption rate profile showed a monoeponential decline on the first day, and the cumulative amount of rhGH absorbed versus time curve exhibited a typical first-order absorption process. The estimated parameters by computer fitting (eqs. 11–14) are listed in Table 2. After the s.c. dose, the peak concentration ($C_{\text{max}}$) of about 2700 ng/ml appeared shortly after the dosing ($T_{\text{max}}$ is about 1 h). The concentration...
returned to its baseline level (1.8 ng/ml) in 2 days. Bioavailability of rhGH can be estimated by \( A_{\text{Aa}} = \frac{C}{\text{Injection dose}} \). The overall and net bioavailabilities by the AUC method are 0.63 and 0.71 (Table 3). Daily rhGH s.c. Dosing. The serum rhGH concentration versus time and the rhGH absorption profiles are shown in Fig. 6. For the first dose, the results showed that first-order absorption was involved. Estimations of parameters by computer fitting (eqs. 15–18) are listed in Table 2. Each s.c. dose had a peak concentration of about 200 ng/ml at 1 h postdose. The accumulation effect of multiple dosing is not seen. After the last dose on day 27, rhGH concentration declined to its baseline value estimated as 2.52 ng/ml. The estimated bioavailability for each dose from computer fitting is 0.86, which is very close to the results from the AUC analysis done for the first dose, where overall and net bioavailabilities are 0.85 and 0.88 (Table 3).

### Pharmacodynamics

The IGF-I concentrations versus time profiles for group A (ProLease rhGH) and group B (single s.c. plus pump) are shown in Fig. 7. The parameters estimated for eqs. 19 and 20 by computer fitting are listed in Table 4. For group A, IGF-I has a baseline value estimated as 310.5 ng/ml. After ProLease rhGH was given, IGF-I gradually increased to 800 ng/ml at about 20 days after dosing and returned to its predose baseline slowly by about 40 days. For group B, the baseline for IGF-I is estimated as 245.4 ng/ml. The IGF-I induction has a maximum value of 750 ng/ml at about 30 days after dosing and returned to the baseline by about 40 days. The estimated \( SC_{50} \) value is 6.8 ng/ml. When the serum

---

**Table 3**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Dose</th>
<th>AUC&lt;sub&gt;total&lt;/sub&gt; (mg)</th>
<th>AUC&lt;sub&gt;base&lt;/sub&gt; (ng/ml·day)</th>
<th>AUC&lt;sub&gt;net&lt;/sub&gt; (ng/ml·day)</th>
<th>F&lt;sup&gt;a&lt;/sup&gt;</th>
<th>F&lt;sub&gt;net&lt;/sub&gt; &lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-term i.v. infusion</td>
<td>0.2392</td>
<td>12</td>
<td>1.6</td>
<td>10.4</td>
<td>1.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.286&lt;sup&gt;d&lt;/sup&gt;</td>
<td>133</td>
<td>1.6</td>
<td>131.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Group A (ProLease)</td>
<td>24.0</td>
<td>550</td>
<td>95.5</td>
<td>454.5</td>
<td>0.51</td>
<td>0.47</td>
</tr>
<tr>
<td>Group B (s.c. plus pump)</td>
<td>24.4</td>
<td>550</td>
<td>71.8</td>
<td>478.2</td>
<td>0.50</td>
<td>0.49</td>
</tr>
<tr>
<td>Group C (single s.c.)</td>
<td>25.9</td>
<td>740</td>
<td>3.7</td>
<td>736.3</td>
<td>0.63</td>
<td>0.71</td>
</tr>
<tr>
<td>Group D (daily s.c.)</td>
<td>0.86</td>
<td>33</td>
<td>2.5</td>
<td>30.5</td>
<td>0.85</td>
<td>0.88</td>
</tr>
</tbody>
</table>

<sup>a</sup> Determined by the trapezoidal rules. AUC<sub>net</sub> = AUC<sub>total</sub> - AUC<sub>base</sub>. AUC<sub>base</sub> is AUC under the baseline value. Time points included for the calculation: day 0 to 74 for groups A and B; day 0 to day 1 for groups C and D. AUC<sub>total</sub> values were reported in Lee et al., 1997.<br>
<sup>b</sup> Overall bioavailability values calculated by AUC<sub>total</sub> were reported in Lee et al., 1997.<br>
<sup>c</sup> Net bioavailability F<sub>net</sub> = \( \frac{(AUC_{\text{test,net}}/Dose_{\text{test}})}{(AUC_{\text{ref,net}}/Dose_{\text{ref}})} \).<br>
<sup>d</sup> As reference i.v. dose.<br>
<sup>e</sup> Only the first dose was included in this analysis.
rhGH concentration is higher than this level, growth hormone can increase IGF-I formation rate by as much as 3.2-fold (estimated \( S_{\text{max}} \) is 2.2). The elimination rate constant for total IGF-I (\( k_{\text{out}} \)) is 0.18 day\(^{-1}\), which means the half-life of total IGF-I is about 4 days. This value should be much longer than the half-life of free IGF-I (Guler et al., 1989; Lieberman et al., 1992).

Simulations for groups C and D were performed using eqs. 19 and 20 and the predicted parameter values. The superposition of our data and the simulated results shown in Fig. 8 indicate that the proposed PK/PD model can describe and predict IGF-I induction under different rhGH-dosing regimens. For group C, the IGF-I has a low baseline (190.8 ng/ml). The peak IGF-I level appeared at 1 to 2 days after dosing and returned to baseline in 5 days. For group D, the accumulation of IGF-I reached its steady state (peak concentration \( \approx 350 \) ng/ml) about 20 days postdosing. Daily injection was stopped at day 27; the IGF-I then declined to its predose level.

The AUEC for total IGF-I induction are listed in Table 5. Group B produced the largest net AUEC value. Group C has the lowest net AUEC value despite being given the highest rhGH dose. Comparing ProLease versus pulsatile injections, results for group A show that monkeys given ProLease rhGH can induce IGF-I more effectively than daily solution injection (group D).

To demonstrate the comparative relationships between rhGH serum concentrations and IGF-I induction, simulations for the four rhGH treatment groups based on the proposed PK/PD model are presented in Figs. 9 and 10. Groups A and B had similar rhGH input schemes in which rhGH was delivered into the system by continuous zero-order inputs. As a result, serum rhGH concentrations were maintained above the SC\(_{50}\) value much longer than the single (group C) or daily (group D) SC injections. ProLease was able to produce a surge of rhGH concentration for about 1.3 days and maintain the level at another steady-state concentration for 16 days. As shown in Fig. 10, IGF-I induction is very effective in that period of time as the rhGH concentrations were higher than the estimated SC\(_{50}\) value. For group B, a peak rhGH concentration is produced by the single s.c. injection followed by a steady-state concentration maintained by the osmotic pump. For 30 days, the rhGH level was kept above the SC\(_{50}\) value producing appreciable IGF-I induction. For group C, although the large single dose of rhGH produced the highest peak concentration among the four groups, the rhGH level fell below the SC\(_{50}\) quickly by 2 days after dosing (Fig. 10). Lowest IGF-I induction was seen in this group (Fig. 10). For group D, daily injections provided pulsatile growth hormone inputs, but rhGH serum concentrations were below SC\(_{50}\) value for about one-third of the day during the daily injection period (Fig. 9). As a result, modest IGF-I induction was observed (Fig. 10). Although each \( C_{\text{max}} \) produced by the daily injection in group D is lower than the \( C_{\text{max}} \) produced by a single injection in group C, group D had a greater IGF-I induction by the end of the treatment due to its accumulative effects.

**Discussion**

The pharmacokinetics of rhGH was assessed in monkeys following two 5-min short-term infusions. A two-compartment-
ment model describes the fast elimination \((CL = 16.1 \text{ ml/min})\) and slow distribution \((CL_{d} = 0.35 \text{ ml/min})\) of this hormone. The pharmacokinetic parameters are similar to values reported by Mordenti et al. (1991) who found \(CL = 14.7 \text{ ml/min}, V_{c} = 199 \text{ ml}, \text{and } V_{ss} = 314 \text{ ml}\). The numerical deconvolution method (Cutler, 1978; Veng-Pedersen, 1988) then allows us to study the input rates for different rhGH delivery systems. Once the input profiles were identified, input parameters (such as absorption rate constant, input rate, and input duration) can be estimated by computer fittings using fixed pharmacokinetic parameters determined in the disposition study.

ProLease provides three different rhGH zero-order deliver rates in monkeys in three periods: an initial surge (4.077 mg/day) for 1.3 days as a loading dose, followed by a moderate rate (0.184 mg/day) for 16 days, and then a low rate (0.063 mg/day) for up to 62 days. This third phase of low rhGH input rate may be due to a slow release of the hormone from the microspheres or to the cross-reactivity with endogenous monkey GH (Lee et al., 1997). Because these estimates are model-dependent, different release rates over different periods for ProLease rhGH would be obtained if more (or fewer) phases for the rhGH release were assumed for the analysis. Our results show that this sustained-release formulation can maintain rhGH concentration above the SC50 value (6.8 ng/ml) for IGF-I induction for 16 days postdosing. Therefore, the AUEC of IGF-I induction for ProLease rhGH is much higher than from single and daily s.c. injections. Similar to ProLease, the osmotic pump delivers rhGH as a zero-order input and induces IGF-I effectively until removal of the pump (30 days).

Two bioavailability values for each rhGH treatment group were calculated by the AUC method (Table 3). The overall bioavailability \((F)\), calculated by the AUCtotal values, represents the bioavailability of the growth hormone without adjusting its endogenous baseline level. The net bioavailability \((F_{net})\) was calculated by the AUCnet values, which were produced exclusively by the hormone treatments. Theoretically, \(F_{net}\) may better estimate the true rhGH bioavailability; however, the variability and uncertainties in the estimations for the endogenous baseline levels may create some modest errors for \(F_{net}\).

The indirect response model applied in the PK/PD analysis provides an excellent characterization of the diverse treatments with a common set of parameters. Using the pharma-

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Dose</th>
<th>Induced Days</th>
<th>AUEC(^{\text{total}})</th>
<th>AUEC(_{\text{base}})</th>
<th>AUEC(_{\text{net}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (ProLease)</td>
<td>24.0</td>
<td>0–60</td>
<td>29,391</td>
<td>18,630</td>
<td>10,761</td>
</tr>
<tr>
<td>Group B (s.c. plus pump)</td>
<td>24.4</td>
<td>0–55</td>
<td>29,100</td>
<td>13,497</td>
<td>15,603</td>
</tr>
<tr>
<td>Group C (single s.c.)</td>
<td>25.9</td>
<td>0–10</td>
<td>2,660</td>
<td>1,908</td>
<td>752</td>
</tr>
<tr>
<td>Group D (daily s.c.)</td>
<td>24.1</td>
<td>0–55</td>
<td>17,735</td>
<td>11,330</td>
<td>6,405</td>
</tr>
</tbody>
</table>

Fig. 9. Simulations of rhGH serum concentrations for the four rhGH treatments. The dashed lines are the rhGH SC50 value (ng/ml) for IGF-I induction estimated by the indirect response model (Fig. 1).

Fig. 10. Simulations of IGF-I induction by four different rhGH treatments using the indirect response model (Fig. 1).
cokineti c profiles of rhGH as forcing functions in the dynamic analysis, IGF-I level is described as a reversible induction process. The use of the slope coefficient $\gamma$ and the definition of the initial conditions of the differential equations allowed the model to better describe the abrupt nature of the response curve and reflect the baseline hormone levels for the response. This approach also allows us to estimate the potency factor $SC_{50}$. This factor provides important information to optimize rhGH delivery systems as it is crucial to maintain rhGH serum concentration above the $SC_{50}$ value to induce IGF-I effectively. We showed that both ProLease rhGH and osmotic pump delivery were able to attain this goal for a lengthy period. Their IGF-I inductions were much more effective than single and daily s.c. injections (as shown in Figs. 9 and 10).

Several studies have tested a similar concept by prolonging serum GH circulating time. When rhGH was coadministered with rhGH-binding protein (rhGHBP) by s.c. injection in HX rats, the GH serum concentration profile had a wider peak with a similar maximum concentration compared with GH alone. As a result, rhGH plus rhGHBP daily injections for 8 days produced better weight gain than GH alone (Clark et al., 1996a). Another study showed that polyethylene glycol-conjugated GH formulations had longer half-lives compared with unconjugated GH in HX rats and produced similar weight gains when polyethylene glycol-GH s.c. injections were given twice in 12 days compared with daily GH injections (Clark et al., 1996b). Furthermore, Fielder et al. (1996) showed that the coadministration of GH and IGF-I in HX rats was more effective for long-term weight gains than GH or IGF-I alone. Therefore, the continuous exposure of GH may promote desirable growth effect resulting from the induction of IGF-I and the presence of the hormone itself. Although our monkey studies showed promising results for IGF-I induction (Johnson et al., 1996; Lee et al., 1997), the effectiveness of long-term ProLease GH treatment in GH-deficient children is now under investigation. The proposed PK/PD model, which characterizes the biological events for GH disposition and the subsequent IGF-I induction profile, may help us better understand the role of this hormone in the future.

The numerical deconvolution method (Cutler, 1978; Veng-Pedersen, 1988) is very useful to identify drug input profiles and estimate the cumulative amount of the drug in the system. There are some assumptions and limitations of this method. For example, rhGH kinetics are assumed to be linear and stationary for the range of rhGH doses given in the study (Table 3). Low interindividual variation of rhGH kinetics is assumed to allow the same rhGH disposition function among different groups of monkeys. To interpret the absorption rate and cumulative amount of GH absorbed, an approximation process was needed to utilize the information in the compartmental analysis. For instance, ProLease rhGH has an irregular input profile in the first 2 days (Fig. 3). A zero-order input process was assumed, and the model predicted steady state to be reached in about 12 h. This is consistent with the release profile obtained from the in vitro study (Johnson et al., 1996). However, the model overestimates the rhGH concentration between days 1 and 1.3. An alternative approach is to use a spline-type polynomial equation to describe the input rate. However, such a method cannot predict the rhGH kinetic profile within differential equations. Our current approach is advantageous in the drug development process because it allows extrapolation.

Short-term i.v. infusion studies clearly showed that rhGH concentrations declined biexponentially; however, this was not evident when s.c. rhGH injections were given. This is common for extravascular administration when $k_e$ is close to $\lambda_g$ (Gibaldi and Perrier, 1982). Since the $k_i$ values (Table 2) for rhGH s.c. injections are lower than the disposition rate constants (Table 1), flip-flop kinetics may occur where terminal slopes are not as steep as the slope in the short-term infusion studies. Protein binding of growth hormone is another important issue. The primary source of serum GHBP is an extracellular domain of growth hormone receptor cleaved from cell membranes (Leung et al., 1987). Under physiological conditions in humans, circulating growth hormone is about 39 to 59% bound to GHBP (Baumann et al., 1988). In a rhGH replacement study in GH-deficient children (Tauber et al., 1993), continuous infusions (0.1 IU/kg daily) for 6 months increased the GHBP level from predose 8.6 ± 3.1% to 22.5 ± 9.8%. The normal range for children is reported as 24.8 ± 1.7% (Tar et al., 1990). Meanwhile, total IGF-I was increased from 134 to 329 ng/ml in the same study. As demonstrated by Clark et al. (1996a), GHBP alters the pharmacokinetics of rhGH, and its dynamic responses need further investigation. Monkey GH and native human GH differ by four amino acids (Li et al., 1986); therefore, it is possible that the tested monkeys will develop antibodies after rhGH is given. Anti-hGH antibodies were detected in only one of four monkeys given ProLease rhGH, and the results suggested that rhGH released from ProLease is no more immunogenic than unformulated protein (Johnson et al., 1996; Lee et al., 1997).

In conclusion, our proposed PK/PD model can describe rhGH pharmacokinetics and total IGF-I induction in monkeys under diverse dosing conditions. ProLease is an effective drug delivery system for continuous growth hormone replacement therapy. Continuous rhGH input delivered by ProLease and osmotic pump is more effective than pulsatile injections in induction of IGF-I, especially when the delivery systems can maintain growth hormone concentration above the $SC_{50}$.

Acknowledgments

We thank Dr. Jorgarao Gobburu (SUNY at Buffalo) for his suggestions for the modeling and data analysis.

References


Send reprint requests to: William J. Jusko, Ph.D., 565 Hochstetter Hall, Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14260. E-mail: wjjusko@acsu.buffalo.edu.