Pharmacokinetics and Pharmacodynamics of a Humanized Monoclonal Antibody to Factor IX in Cynomolgus Monkeys¹

LISA J. BENINCOSA, FUNG-SING CHOW, LEEANN P. TOBIA, DEBORAH C. KWOK, CHARLES B. DAVIS, and WILLIAM J. JUSKO

Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York (F.-S.C., W.J.J.); and SmithKline Beecham Pharmaceuticals, Drug Metabolism and Pharmacokinetics, King of Prussia, Pennsylvania (L.J.B., F.-S.C., L.P.T., D.C.K., C.B.D.)

Accepted for publication October 25, 1999   This paper is available online at http://www.jpet.org

ABSTRACT

The pharmacokinetics and pharmacodynamics (PK/PD) of a humanized anti-Factor IX IgG1 monoclonal antibody (SB 249417, FIX mAb) were studied in Cynomolgus monkeys. Single i.v. bolus doses of 1, 3, or 10 mg/kg of FIX mAb were administered. The total FIX mAb concentration, activated partial thromboplastin time (aPTT), and Factor IX activity were monitored for up to 4 weeks after dosing. In the monkey, FIX mAb had a plasma clearance of 0.6 ml/h/kg and a steady-state volume of distribution of approximately 70 ml/kg. The elimination phase half-life (3.8 days) was considerably less than other humanized IgG1 mAbs in the monkey, for which there is no binding to endogenous antigen. The suppression of Factor IX activity and the prolongation of aPTT were rapid and dose dependent. The time for aPTT values to return to basal levels (25–170 h) increased with increasing dose. A mechanism-based PK/PD model consistent with the stoichiometry of binding (2:1) was developed to describe the Factor IX activity and aPTT response time course. The model incorporated Factor IX synthesis and degradation rates that were interrupted by the sequestration of Factor IX by the antibody. aPTT values were related to free Factor IX activity. This model was able to describe the PD profiles from the three dose levels simultaneously. The estimated Factor IX half-life was 11 h and the third-order association rate constant was 3.96 × 10⁵ μM⁻² h⁻¹. The PK/PD modeling was useful in summarizing the major determinants (endogenous and antibody-ligand binding) controlling FIX mAb-related effects.

Anticoagulant therapy is important for treatment of various vascular disorders including coronary artery thrombosis, deep venous thrombosis, pulmonary embolism, and peripheral arterial occlusion. The types of agents commonly in use include heparin, which acts by binding to antithrombin III (ATIII) and inactivating a number of coagulation enzymes including thrombin (IIa) and Factor Xa (Samama et al., 1996); warfarin, which inhibits vitamin K-dependent synthesis of Factors II, VII, IX, and X and proteins C and S (Kessler, 1991); and antiplatelet drugs (such as aspirin, thrombin inhibitors, ADP inhibitors, and GPIIb/IIIa antagonists) (Schrör, 1995).

The coagulation system can be activated by two separate pathways: the tissue (extrinsic) and contact factor (intrinsic) pathways. Such activation results in the production of thrombin and subsequently the formation of fibrin (Davie, 1995; Mannucci, 1994). SB 249417 (FIX mAb) is a humanized monoclonal antibody (mAb) (IgG1) with specificity for human Factor IXa/IXa (Kₐ = 12 nM). FIX mAb recognizes monkey Factor IX with comparable affinity as the human antigen. Antibody binding inhibits the activation of the zymogen, Factor IX, and also blocks the activity of Factor IXa on Factor X, the subsequent enzyme in the clotting cascade. Inhibition or removal of Factor IX by coupling to an antibody represents a novel approach to anticoagulant therapy. In vitro, the anti-Factor IX mAb extended the activated partial thromboplastin time (aPTT) to a plateau of 60 and 80 s (rat and human reference plasma, respectively) over the concentration range of 100 to 1000 nM (Feuerstein et al., 1999). In contrast, high levels of heparin prolonged clotting times indefinitely (Feuer-

ABBREVIATIONS: ATIII, antithrombin III; GPIIb/IIIa, glycoprotein IIb/IIIa receptor; mAb, monoclonal antibody; FIX mAb, SB 249417; Ab, free antibody concentration; AbFIX₂, SB 249417-Factor IX complex; Cᵢ, total antibody concentration; aPTT, activated partial thromboplastin time; PK, pharmacokinetic; PD, pharmacodynamic; FIX, free Factor IX; kₑ₀, first-order elimination rate constant; kₑ₀, systemic elimination rate constant; kₑᵢ, first-order elimination rate constant; kₑᵢ, systemic elimination rate constant; kₑᵢ, degradation half-life; Vₛ, Central volume of distribution; Vₚₛ, steady-state volume of distribution; kₛᵢ, the zero-order Factor IX synthesis rate constant; kₖᵢ, third-order association rate constant; kₖᵢ, the first-order dissociation rate constant.
stein et al., 1999). The murine parent anti-human Factor IX antibody has been shown to prevent thrombosis in a rat arterial thrombosis model (Feuerstein et al., 1999). Furthermore, compared with aspirin and heparin, this murine parent antibody demonstrated superior anti-thrombotic efficacy in vivo with limited extension of aPTT and blood loss.

In this study, the pharmacokinetics (PK) of FIX mAb were characterized in monkeys after i.v. bolus administration of three dose levels. Pharmacodynamic (PD) measurements included the time course of Factor IX activity and aPTT. The aPTT, a well accepted indicator of deficiencies in the intrinsic pathways of coagulation (i.e., factors VIII, IX, XII, and XI), was chosen as the PD endpoint for the effects of FIX mAb (Rodvold and Friedenberg, 1989). The objective was to establish the underlying relationship between drug administration factors (dose, frequency, route) and the time course of pharmacologic response. A mechanism-based, PK/PD model was developed based on concepts of antibody-ligand interaction and synthesis and degradation of endogenous Factor IX.

Materials and Methods

Chemicals. Humanized anti-Factor IX mAb was expressed in Chinese hamster ovary cells and purified to homogeneity. Recombinant human Factor IX was obtained from Genetics Institute (Andover, MA). Mouse anti-human IgG1 mAb (clone HP6069) was purchased from Zymed Laboratories (San Francisco, CA). Actin-activated cephaloplatin reagent and 0.02 M calcium chloride were purchased from Dade Diagnostics (Aguada, Puerto Rico). Normal hemostasis reference plasma was obtained from American Diagnostics (Greenwich, CT). All other chemicals were of reagent grade or better.

Animals. Male Cynomolgous monkeys (3.7–6.6 kg), supplied by Covance (Denver, PA) or by Primate Products (Miami, FL), were used in this study. Monkeys were quarantined for at least 3 months before treatment and were screened for tuberculosis, parasites, and any clinical pathologic abnormalities. The monkeys were housed individually in stainless-steel cages in a controlled environment with a 12-h light/dark cycle. Filtered tap water was available ad libitum and food was provided twice daily. On the dosing day, animals had catheters placed in the cephalic vein for dosing and in the saphenous vein for blood sample collection. Animals were restrained in Plas-Lab Medium Restrainer chairs (Plas Labs Inc., Lansing, MI) for up to 4 h on the dosing day. Animals were slightly sedated (10 mg/kg ketamine, i.m.) for blood sample collection after the dosing day.

Dosing. Groups of monkeys (n = 2 per dose group) received single i.v. bolus doses of 1, 3, or 10 mg/kg of FIX mAb via the cephalic cannula. The dose was delivered over 1 min in a volume of ~2 ml/kg followed by a saline flush of the cannula.

PK Assessment. Blood samples (1 ml per time point) were collected at 0, 0.1, 4 (except 10 mg/kg group), 8, 24, 48, 72, 96 h, and 7, 10.5, 14, 21, and 28 days. Plasma concentrations of FIX mAb were determined using an electrochemiluminescent immunoassay based on the binding of FIX mAb to recombinant human Factor IX. Briefly, heparinized plasma samples were diluted 10-fold then added to biotinylated Factor IX and streptavidin-conjugated paramagnetic beads. The beads were isolated by magnetic separation and then mixed with ruthenium-labeled mouse anti-human mAb specific for the CH2 domain of human IgG1 (Hamilton and Morrison, 1993). Electrochemiluminescent response was recorded with an Origin Analyzer (Igen, Inc., Gaithersburg, MD). Standard curves ranged from 50 to 4250 ng/ml FIX mAb in heparinized monkey plasma. The lower limit of quantification of the assay was 50 ng/ml (10 μl plasma). Assay precision was within 6.5% over the calibration range, and average bias was <10.5%.

Antibody binding to Factor IX is dependent on calcium ion. In the immunoassay, as the plasma sample is diluted to a concentration within the calibration range, the calcium in plasma is diluted, prompting dissociation of antigen-antibody complexes. In the last step of the assay, calcium is added back in the presence of excess recombinant human Factor IX. Thus the assay measures total antibody concentration based on its ability to bind to the human antigen. A positive assay response is indicative of a species containing an antigen-combining site and the hinge epitope of the constant region of the heavy chain domain 2. The presence of these two features would suggest that the analyte measured is intact mAb.

PD Assessment. Both Factor IX activity and aPTT served as the PD markers for FIX mAb. Blood samples (0.5 or 1 ml) were collected at the same time points as described for PK assessments. The total volume of blood taken during the first week after dose administration (for PK and PD) was <15% of the total blood volume. If analysis indicated that aPTT had returned to baseline for two consecutive sampling times, further blood sampling for aPTT was not performed.

Dosing was determined in each animal at the three dose levels; Factor IX activity was determined in the 1 and 3 mg/kg dose groups. Factor IX activity as not determined after administration of 10 mg/kg. The aPTT was measured using a BBL Fibrometer (Becton Dickinson, Cockeysville, MD). The assay procedure employed was as described by one manufacturer of assay reagents (Baxter Diagnostics, McGaw Park, IL). Control normal hemostasis reference plasma was analyzed with authentic samples. Factor IX activity was measured using an Electra 1000C Automatic Coagulation Timer (Medical Laboratory Automation, Pleasantville, NY). A six-point standard curve (1:10 to 1:320) was prepared for Factor IX using a citrated plasma pool from healthy stock monkeys. The lower limit of quantification was 3% Factor IX activity. Plasma samples were diluted 1:10 in Owen’s Veronal buffer (Dade Diagnostics) before testing. Human immunoabsorbed Factor IX-deficient plasma (Dade Diagnostics) was used as the substrate plasma.

PK Modeling. Biexponential fitting of plasma concentrations (Ct) versus time (t) (eq. 1) was performed by weighted (1/y2) nonlinear regression analysis (Allen, 1990).

\[ C_t = C_1 \cdot e^{-\lambda_1 \cdot t} + C_2 \cdot e^{-\lambda_2 \cdot t} \]  
(1)

where C1 and C2 are intercepts and λ1 and λ2 are disposition slopes. The percentages of total area under the curve in the λ1 and λ2 disposition phases were calculated by the method of separate exponentials (Shand et al., 1981). Parameters of the two-compartment model were then calculated using traditional methods (Gibaldi and Perrier, 1982).

PD Modeling. The PD model shown in Fig. 1 was developed to relate FIX mAb plasma concentrations to Factor IX activity. Factor IX activity was used as a measure of free Factor IX. In general, the PD model assumes natural synthesis and degradation of Factor IX that is interrupted by the sequestration of Factor IX by the antibody, FIX mAb. Differential equations proposed to describe the rates of change of free Factor IX (FIX) and the rate of change of FIX mAb-Factor IX complex (AbFIX2) were:

\[ \frac{dFIX}{dt} = k_{syn} - k_{deg} \cdot FIX - [2 \cdot k_{on} \cdot (C_1 - AbFIX2) \cdot FIX^2] + [2 \cdot k_{off} \cdot AbFIX2] \]  
(2)

\[ \frac{dAbFIX2}{dt} = [k_{on} \cdot (C_1 - AbFIX2) \cdot FIX^2] - [k_{off} \cdot AbFIX2] \]  
(3)

where ksyn is the zero-order Factor IX synthesis rate constant, kdeg is the first-order Factor IX degradation rate constant, C1 is the total FIX mAb concentration, [AbFIX2] is the free FIX mAb concentration, koff is the third-order rate constant for the binding of free FIX AbFIX2...
PK/PD Model

mAb to Factor IX, $k_{off}$ is the first order rate constant for the dissociation of FIX mAb from Factor IX, and $k_{el}$ is the first-order FIX mAb-Factor IX complex removal rate constant.

This model assumed that the stoichiometry of binding of Factor IX to FIX mAb was 2:1, consistent with the theoretical bivalency of IgG antibodies.

To describe this binding, the model was operated in molar concentrations. The total FIX mAb molar concentration in plasma ($C_t$) was described with eq. 1. The average PK parameter values for each dose group were used in the PK/PD modeling. Free FIX mAb plasma concentration was obtained as the difference between $C_t$ and AbFIX2. In order to convert Factor IX activity (% into molar concentration ($\mu$M), 100% Factor IX activity was assigned a concentration of 10 $\mu$g/ml (0.182 $\mu$M using a molecular weight of 55,000), which represents the physiologic concentration of Factor IX in the monkey.

Equation 3 describes the rate of change of AbFIX2 in plasma. The reversible binding process was controlled by $k_{on}$ and $k_{off}$. The value of $k_{off}$ was fixed to 1.48 h$^{-1}$ as determined from in vitro experiments. The elimination process of complex was described as a first-order elimination rate constant ($k_{el}$) equal to the rate constant of the elimination process ($k_2$) for total antibody.

Observed aPTT values were related to the measured Factor IX activity by a log-log relationship (Brandt et al., 1990). A linear function (eq. 4) was fitted to the data (1 and 3 mg/kg groups)

$$\log \text{ aPTT} = -b \cdot \log \text{ FIX} + c$$

where $b$ is the slope and $c$ is the intercept. Factor IX activity and aPTT values from the 1 and 3 mg/kg groups were used for this linear regression.

All derived equations were fitted to the Factor IX activity and aPTT data using Adapt II release 4 (D’Argenio and Schumitzky, 1979). Assuming that the additive error is normally distributed, Maximum Likelihood Estimator and a general variance model was used. Initial values for parameters were varied to examine the stability of the model estimates. Data from all dosing groups were fitted simultaneously to generate estimates of $k_{syn}$ and $k_{deg}$. An initial steady-state condition was defined in the model: baseline Factor IX activity ($\text{FIX}_0$) = $k_{syn}/k_{deg}$. The Factor IX degradation half-life ($t_{1/2,\text{deg}}$ = 0.693/$k_{deg}$) was generated as a secondary parameter.

Results

Pharmacokinetics. The PK of FIX mAb were characterized after single i.v. bolus doses of 1, 3, or 10 mg/kg. Mean concentration-time profiles are depicted in Fig. 2. Individual and mean PK parameters derived from analysis of the plasma concentration-time data are summarized in Table 1. A biphasic decline in plasma concentrations was observed for all doses in these monkeys. The dominant terminal disposition phase was characterized with a mean half-life of 91 h. This accounted for an average of 84% of the area under the plasma concentration versus time curve. The shorter disposition phase was characterized by a half-life of approximately 12 h (range of 6–19 h). The observed maximal plasma concentrations were consistent with a central distribution volume ($V_c$) equal to the plasma volume (Davies and Morris, 1993). Steady-state volume of distribution ($V_{ss}$) was estimated to be 69 ± 19 ml/kg. Plasma clearance was low and averaged 0.6 ml/h/kg. The estimated mean disposition slope ($\lambda_2$) was 0.00789 h$^{-1}$ and the calculated mean systemic elimination rate constant ($k_{syn}$) was 0.0160 h$^{-1}$ (Table 1).

Pharmacodynamics. The relationship between log aPTT and log Factor IX activity was well described, with 93% of the total variation in aPTT explained by the regression (coefficient of determination = 0.93) (Fig. 3). Fitting eq. 4, the estimated slope constant ($b$) was 0.33 and intercept ($c$) was 1.96.

The Factor IX activity and aPTT time course are shown in Figs. 4 and 5. After the 1-mg/kg dose of FIX mAb, Factor IX activity decreased to a minimum of 16 to 27% at the earliest sampling time (5 min) and recovered gradually within 24 to 48 h. After the 3-mg/kg dose of FIX mAb, Factor IX activity decreased to below detectable levels (<3%) at the earliest sampling time and gradually returned to baseline over the next 72 h. Generally, aPTT increased from a predose value of 20 s to a value 2- to 3-fold greater than baseline at the earliest time after drug administration. The maximum aPTT observed was approximately 60 s in both the 3- and 10-mg/kg dose groups; drug concentrations at this time point were approximately 80 and 260 $\mu$g/ml. The duration of increase in aPTT was dose dependent: times for aPTT returning to baseline were within 24 h for the 1 mg/kg group, 24 to 72 h for the 3-mg/kg group and 168 h for the 10-mg/kg group. The time course of normalization of aPTT was similar to the time course of recovery of Factor IX activity.

Use of the PK/PD model for fitting Factor IX and aPTT data are shown for the three dose levels in Figs. 4 and 5. The estimated Factor IX synthesis rate ($k_{syn}$) was 0.0113 $\mu$/h or 6.23%/h. The third-order association rate constant ($k_{on}$) was 3.96 $\times$ 10$^4$ $\mu$M$^{-2}$ h$^{-1}$. The resulting degradation $t_{1/2,\text{deg}}$ for Factor IX was 11 h. Figure 6 depicts the concentration-time profiles of the total ($C_t$), complex (AbFIX2), and the free ($C_t$ - AbFIX2) FIX mAb calculated from the PK/PD model. After the 10-mg/kg dose, antibody-antigen complex concentrations...
immediately increased from an initial value of 0 to 0.091 μM. Nearly all of the endogenous Factor IX soon became complexed because a large excess of antibody was administered. Complex concentrations then further increased as a result of continued synthesis of Factor IX. For the 10-mg/kg dose, this required approximately 120 h, until all antibody was in the complexed form. After 120 h, the values of $Ab_{FIX}^2$ and $C_t$ were essentially the same (6.5% difference in concentration). Thereafter, complex and total antibody concentration remained the same and both declined at the same rate. Thus at later times, the concentration-time profile of total antibody is comprised of essentially all inactive complex. Similar trends were apparent for the two lower doses with the magnitude and time of maximal accumulation of complex a function of administered dose. At 3 mg/kg, $Ab_{FIX}^2$ and $C_t$ were very similar at approximately 40 h after administration, whereas at 1 mg/kg, $Ab_{FIX}^2$ and $C_t$ were very similar by 12 h.

**Discussion**

The analysis of plasma FIX mAb concentration versus time data showed the kinetics to be linear. The short distribution phase and the values of $V_{ss}$ and $V_c$ are similar to other humanized antibodies (for example, Gobburu et al., 1998). With a $V_{ss}$ similar to blood volume and a kinetically dominant terminal disposition phase, the distribution of FIX mAb outside of the systemic circulation was minimal. Because the FIX mAb immunoassay measured the total concentration in blood, the elimination phase half-life (3.8 days) reflected the elimination rate of the FIX mAb-Factor IX complex. This was consistent with results of studies in the rat, in which concent-
trations of antibody-antigen complex were measured directly (Davis et al., 1999).

The concentration and distribution of the target antigen can have a significant impact on the PK of mAb as well as on PK linearity (Davis and Bugelski, 1998; Davis et al., 1996; and Mould et al., 1999). It is similarly important to consider immunoassay specificity (for example, whether one is measuring the concentration of bound, free, or total) when comparing the PK behavior of different mAb. Antibodies that target endogenous molecules present in very low concentrations tend to have longer elimination half-lives (Zia-Amirhosseini et al., 1999), closer to that reported for antibodies targeting exogenous molecules (for example, Davis et al., 1995). The results of the present investigation and our studies of FIX mAb in the rat (Davis et al., 1999) indicate that high concentrations of soluble, circulating Factor IX antigen have a major impact on the disposition of this humanized mAb. The elimination half-life of antibody-antigen complex (4 days) is long but considerably shorter than what might be expected for free antibody in the absence of the coagulation factor.

Human IgG1 consists of one Fc and two Fab domains. The amino terminus of each Fab domain forms an antigen-binding site. In the simplest condition, one can assume that a single molecule (i.e., FIX mAb) circulates in the blood system with a capacity of binding two antigen (i.e., Factor IX) molecules. Based on this understanding, a mass-balance, mechanistic PD model was developed to describe the interaction of FIX mAb and Factor IX in blood. To achieve mass balance in the tri-molecular reaction, a third-order association rate constant ($k_{on}$) and a power of two on the free Factor IX were used to describe the binding kinetics of one antibody with two Factor IX molecules (eq. 2). Additionally, the rate of change of free Factor IX was described with association and dissociation rates twice the $k_{on}$ and $k_{off}$ of complex to account for the fact that each molecule of complex contained two molecules of Factor IX.

To describe the mechanistic properties of FIX mAb, the PK/PD model was developed to describe the change of free Factor IX and its complex concentration in blood. aPTT has been well accepted as a clinically relevant measurement of blood clotting and is widely used for detection of any abnormality of the intrinsic pathway in the coagulation system (Rodvold and Friedenberg, 1989). Therefore, the model was expanded to include aPTT data at all dose levels. The mechanistic relationship between Factor IX concentration and
aPTT is not well understood. The aPTT and Factor IX activity has been described as an inversely proportional log-log relationship (Brandt et al., 1990). With this log-log relationship, the Factor IX activity and aPTT values of all three doses were described simultaneously. It is important to note that the log-log relationship does not impose any dose-limiting prolongation of aPTT at low Factor IX activity as was demonstrated in vitro.

The PK/PD model predicted a dose-dependent rebound of Factor IX activity and rapid return phases. Although it underestimated the rebound of both doses, the model has the essential features that are present in the observed data. The model also described the dose-dependent normalization of aPTT. Using the log-log relationship, aPTT was well described at Factor IX activity values above 5%. However, quantitation of this relationship at very low Factor IX concentrations was limited by the assay sensitivity. Therefore, an upper limit of aPTT was not specified when Factor IX activity was lower than the detection limit (3% Factor IX activity). At 10 mg/kg, Factor IX activity is predicted to be <5% for the first 40 h. During this period, overestimation of the aPTT was evident as the log-log relationship predicts that lower Factor IX activity will cause continually increasing aPTT. However, the observation that individual aPTT values in this study did not exceed 65 s, even when Factor IX activity was nonquantifiable, is consistent with the limited increase in aPTT observed with increasing antibody concentration in vitro (Feuerstein et al. 1999) and the high specificity of this mode of intervention.

Immediately after dosing of all three dose levels, nearly all of the circulating free Factor IX was bound to FIX mAb. This is shown as the same magnitude of the initial rise in simulated complex profiles in Fig. 6. After this rise in complex concentration, the formation rate of complex is determined by the production rate of free Factor IX in blood. Because there is a continuous production of free Factor IX with no further input of free FIX mAb, available free mAb in blood is slowly converted into bound form. This results in a dose-dependent accumulation of complex over time with the largest dose having the highest concentration of complex and requiring the longest time until all of the free antibody is in complex form. Thereafter, inactive complex concentrations

Fig. 6. Simulated FIX mAb concentration-time profiles. Total FIX mAb (Ct) (solid lines), FIX mAb-Factor IX complexes (AbFIX) (dotted lines), and free FIX mAb (broken lines).
decline over time. The dose-dependent accumulation of FIX mAb-Factor IX complex in the rat was substantiated by direct measurement of complex by Western Blot (Davis et al., 1999).

The PK/PD model was also assessed under the conditions where the elimination process of complex ($k_{el}$) was equal to the systemic elimination rate constant for total antibody ($k_{10}$). However, this model was not able to describe the rebound of the Factor IX activity. In addition, the predicted time to return to the baseline (100%) for the 3-mg/kg dose was much longer than observed (data not shown). These results suggest that the elimination rate constant of complex is closely related to the disposition slope of the total FIX mAb concentration profile.

In conclusion, this study presents the PK and PD of a novel humanized anti-Factor IX antibody in monkeys. The distribution of this antibody is mainly in the blood circulation. The elimination of this antibody is dependent on its binding with endogenous Factor IX and the removal rate of the AbFIX2 complex. A mass-balance, mechanism-based PK/PD model with stoichiometry of the binding processes was used. This model was able to describe the dose-dependent effect of FIX mAb on the suppression of Factor IX activity and the prolongation of aPTT response. The present model was developed on the concept of antibody-ligand interaction and should be generally applicable to the study of response profiles of therapeutic mAbs.

Acknowledgments

We gratefully acknowledge Dr. Michael Blackburn (Structural Biology, SmithKline Beecham), Dr. Larry Greller and Dr. Carolyn Cho (Bioinformatics, SmithKline Beecham) for helpful discussions, and Karen Lynch and Teresa Sellers for the Factor IX activity measurements.

References


Send reprint requests to: Lisa J. Benincosa, PhD, SmithKline Beecham Pharmaceuticals, Drug Metabolism and Pharmacokinetics, 709 Swedeland Rd., P.O. Box 1539, King of Prussia, PA 19409. E-mail: Lisa_J_Benincosa@sbphrd.com