약제학회지(2009), 제 39 권 제 5 호
J. Kor. Pharm. Sci., Vol. 39, No. 5, 373-379 (2009)

Pharmacokinetic-Pharmacodynamic Modeling of a Direct Thrombin Inhibitor, Argatroban, in Rats

Eun Hye Park[1], Beom Soo Shin[2], Chi Ho Yun[3], Mann Hyung Lee[2] and Sun Dong Yoo[4†]

[1]*Korea Food & Drug Administration, Seoul, Korea*
[2]*College of Pharmacy, Catholic University of Daegu, Gyeongsan-si, Gyeongbuk, Korea*
[3]*Choongwae Pharm. Co., Hwasung, Gyeonggi-do, Korea*
[4]*College of Pharmacy, Sungkyunkwan University, Suwon, Gyeonggi-do, Korea*

(Received September 28, 2009 · Revised October 1, 2009 · Accepted October 9, 2009)

ABSTRACT – This study was conducted to develop a pharmacokinetic-pharmacodynamic (PK/PD) model of a direct thrombin inhibitor, argatroban to predict the concentration-effect profiles in rats. Argatroban was i.v. injected to rats at 0.2, 0.8 and 3.2 mg/kg doses (n = 4-5 per dose), and plasma drug levels were determined by a validated LC/MS/MS assay. The pharmacokinetics of argatroban was linear over the i.v. dose range studied. The thrombin time (TT) and the activated partial thromboplastin time (aPTT) were measured in rat plasma and they were found to linearly increase with increasing the dose. A 2-compartment pharmacokinetic model linked with an indirect response pharmacodynamic model was successfully utilized to evaluate the drug concentration-response relationship.

Key words – Argatroban, Pharmacokinetics, Pharmacodynamics, Thrombin time, Activated partial thromboplastin time

Argatroban [(2R,4R)-4-methyl-1[N^2-(3-methyl-1,2,3,4-tetrahydro-8-quinoline-sulfonyl)-1-arginyl]-2-piperidinecarboxilic acid] is a L-arginine derivative direct thrombin inhibitor. It binds to the catalytic active site of a thrombin molecule by a reversible interaction.[1)] The thrombin time (TT), prothrombin time (PT) and activated partial thomboplastin time (aPTT) have been measured in human, dog, rabbit and rat plasmas to determine the anticoagulant effects of argatroban.[2)] Animal studies in rats, rabbits and dogs have shown that argatroban exhibits various activities in thromboembolic related diseases. Activities in venous and arterial thrombosis, bleeding, thrombolysis, acute myocardial infarction, cerebral ischemia, infarction, throbmoembolism, disseminated intravascular coagulation and peripheral arterial occlusion have been examined. Most of the tests show that argatroban has either comparable or more effective activity compared to other anticoagulants. Argatroban has been approved by FDA in the use of prophylaxis or treatment of heparin-induced thrombocytopenia (HIT) and HITTS (HIT with thrombosis).[3)]

As with most other peptidomimetic thrombin inhibitors, argatroban exhibits high systemic clearance, short plasma half-life, low bioavailability and extensive hepatic extraction. The extent of protein binding is low in human plasma (54%) with 20% to albumin and 34% to a_1-acid glycoprotein.[3)] It has an elimination half-life of 39-51 min and systemic clearance of 0.3 L/hr/kg (5 ml/min/kg) with minimal effect on pharmacokinetic parameters with the changes in age, gender and renal function.[4)] It is extensively metabolized in the liver by CYP 3A4/5. The major metabolite M1 is found in plasma with having 30% of antithrombotic activity to that of the parent compound. Other metabolites M2 and M3 are found in low quantities in human urine. In drug interaction studies with a potent CYP 3A4 inhibitor, erythromycin, the pharmacokinetics does not change, suggesting that the hepatic metabolism is not the major elimination pathway of argatroban.[5)]

In the present study, the pharmacokinetic and pharmacodynamic parameters of argatroban were determined in rats after i.v. injection at 0.2, 0.8 and 3.2 mg/kg doses. A 2-compartment pharmacokinetic model linked with an indirect response pharmacodynamic model was used to evaluate the concentration-response relationship.

Materials and Methods

Materials

Argatroban and L-375,378 (internal standard) were obtained from C&C Research Laboratories (Hwasung, Korea). The AccuClot™ thrombin time reagent (bovine thrombin, 3-4 NIH units/ml), Alexin™, and calcium chloride solution (0.02 mol/L)

†본 논문에 관한 문의는 이 저자에게로
Tel : 031)290-7717, E-mail : sdyoo@skku.ac.kr
DOI : 10.4333/KPS.2009.39.5.373

were purchased from Sigma-Aldrich-Verwaltungs GmbH (Lemgo, Germany). Ammonium acetate, diethyl ether, and ethanol were purchased from Sigma (St Louis, MO, USA) and methanol and acetonitrile (HPLC grades) were from Merck KGaA (Darmstadt, Germany). Dimethyl sulfoxide and water were obtained from Kanto Chemical Co. (Tokyo, Japan) and J.T. Baker (Phillipsburg, NJ, USA), respectively.

Intravenous Injection Study

Male Sprague-Dawley rats (250-350 g) were obtained from Hanlim Animal Co. (Hwasung, Korea) and kept in plastic cages with free access to standard rat diet (Samyang, Seoul, Korea) and water. The animals were maintained at a temperature of 22-24°C with 12 hr light-dark cycle and relative humidity of 50 ± 10%. Argatroban dissolved in a mixture of DMSO:saline (1:9) was injected into the tail vein at 0.2, 0.8 and 3.2 mg/kg doses (n= 4-5 per dose). Each rat was restrained on a Naigai CFK animal restrainer (Tong Yang Co., Tokyo, Japan) at each sampling time, and venous blood samples were collected from the jugular vein at 0, 5, 15, 30, 45 min, and 1, 2, 4 and 6 hr and immediately preserved in ice. Plasma samples were harvested by centrifugation at 10,000 rpm (Micro 17R, Hanil Co., Inchon, Korea) for 5 min and stored at -20°C until drug analysis. The plasma argatroban concentration-time data was subjected to a compartmental analysis using the nonlinear least-squares regression program WinNonlin (Pharsight, Cary, USA). For the determination of TT and aPTT, blood samples were collected in disposable syringes treated with trisodium citrate dehydrate solution (1 M), and plasma samples were harvested by centrifugation (Micro 17R, Hanil Co., Inchon, Korea) at 10,000 rpm for 5 min and stored at -20°C until analysis.

Hemolytic Activity of Various Solvents as Dosing Vehicles

The hemolytic activity of various solvents as dosing vehicles, e.g., isotonic saline, distilled water (DW), DMSO, mixtures of DMSO:saline (ratios 1:9 and 2:8), DW:polyethylene glycol (PEG) 200:ethanol (3:6:1), and DW:PEG 200:DMSO (3:6:1), were investigated as described previously.[6] To fresh rat blood (270 μl), 30 μl of each prepared solution was added and gently mixed. The samples were incubated at 37°C for 90 min and centrifuged at 10,000 rpm (Micro 17R, Hanil Co., Inchon, Korea) for 10 min. A portion (50 μl) of the upper layer of the centrifuged sample was added to 150 μl of isotonic saline. The absorbance of prepared samples was measured at the 405 nm with blank saline as the 100% reference solution and blood as the negative control solution.

Drug Analysis

Plasma argatroban concentrations were determined by a validated LC/MS/MS assay method. For extraction, the internal standard solution (500 μl, L-375,378 500 ng/ml) was added to each plasma sample (50 μl) in Falcon® polypropylene conical tubes (Becton Dickinson, Franklin Lakes, NJ, USA) and mixed on a vortex mixer for 10 sec. The samples were extracted with diethyl ether (2.5 ml) on a multi-vortex mixer for 10 min followed by centrifugation at 3,500 rpm (VX-2500, VWR, West Chester, PA, USA) for 10 min. The upper organic layer was transferred to a clean borosilicate test tube and evaporated (Heto Maxi Dry Plus, Thermo Electron Corp. San Jose, CA, USA). The residue was dissolved in the mobile phase (150 μl) and mixed on a vortex mixer for 10 min. The solution was transferred to an injection vial and a portion (30 μl) was injected into LC/MS/MS.

HPLC analysis was performed using an Agilent 1100 series HPLC system consisting of a binary pump, an autosampler, a thermostated column compartment and a vacuum degasser (Agilent Technologies, Palo Alto, CA, USA). Chromatographic separations were achieved using an Atlantis C_{18} column (150 x 2.1 mm i.d., 5 mm, Waters Corp., Milford, MA, USA). The isocratic mobile phase consisted of methanol and deionized water containing ammonium acetate (10 mM) (70:30 v/v). The flow rate of the mobile phase and the column oven temperature were 0.2 ml/min and 40°C, respectively. The total run time was 8 min, with the first and last 2 min of chromatographic runs were eluted to waste using a 10-port switching valve (Valco, Houston, TX, USA). The HPLC system was coupled to an API 3000 LC/MS/MS system equipped with a turbo ion spray ionization source (AB MDS Sciex, Foster City, CA, USA). The turbo ion spray ionization source was operated in a negative mode. The curtain, nebulizer, and turbo gas (nitrogen) pressures were set at 10, 9, and 90 psi, respectively. The turbo gas temperature was set at 350°C, and the ion spray needle voltage was adjusted to 5500 V. The mass spectrometer was operated at a unit resolution for both Q1 and Q3 in the multiple reaction monitoring (MRM) mode with a dwell time of 300 ms in each transition. The transition of the precursors to the product ion was monitored at 509.4→383.9 for argatroban and 407.3→120.9 for L-375,378. The collision energy was set at 33 eV for both argatroban and L-375,378. Data acquisition was preformed with the Analyst 1.4 software (AB MSD Sciex, Toronto, Canada).

Calibration curves were prepared by spiking the blank rat plasma (100 μl) with 20 μl of the argatroban standard solution at concentrations of 5, 10, 50, 100, 500, 1,000, 5,000, and 10,000 ng/ml. The calibration curves were constructed by the

weighted regression method (1/x) of peak area ratios of argatroban to internal standard vs. actual concentrations. The linearity of the calibration curves was validated with 5 different calibration curves. The lower limit of quantification (LLOQ) was defined as the lowest plasma argatroban concentration that yielded a signal-to-noise (S/N) ratio >10, with acceptable accuracy and precision (<20%). The precision of the assay was expressed as the coefficient of variation (%CV) of each concentration, and the accuracy was expressed as the percentage of mean calculated vs. actual concentrations. Intra- and inter-day assay variability were determined by assaying drug concentrations at 5, 10, 50, 100, 500, 1,000, 5,000 and 10,000 ng/ml.

Measurement of TT and aPTT

TT and aPTT were determined in rat plasma by a selective multi-channel hemostasis analyzer AMAX 190 Plus (Sigma Diagnostics, St. Louis, MO, USA). The frozen samples were melted in ice and incubated for 30 min prior to determination. The AccuClot™ thrombin time reagent (bovine thrombin, 3-4 NIH units/ml), Alexin™, and calcium chloride solution (0.02 mol/l) (Sigma-Aldrich-Verwaltungs GmbH, Lemgo, Germany) were preincubated inside AMAX 190 before determining TT and aPTT in the plasma. The clotting time beyond 600 sec were outside of the linear range and shown as not coagulated.

PK/PD Modeling

The schematic representation of the pharmacokinetic-pharmacodynamic model for argatroban is shown in Figure 1. The argatroban concentration-time profiles were fit to a 2-compartment model:

$$\frac{dC_p}{dt} = k_{21} \times C_t \times \frac{V_t}{V_p} - k_{12} \times C_p - k_{10} \times C_p \quad \text{(Eq.1)}$$

$$\frac{dC_t}{dt} = k_{21} \times C_p \times \frac{V_p}{V_t} - k_{12} \times C_t \quad \text{(Eq.2)}$$

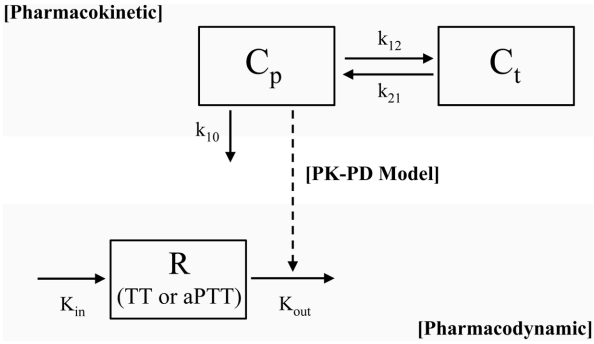

Figure 1—Schematic representation of the PK/PD model used to describe the pharmacokinetics and pharmacodynamics of argatroban in rats.

where C_p and C_t represent drug concentrations in the central and peripheral compartments, respectively, and V_p and V_t are the volumes of distribution in the central and peripheral compartments, respectively. The concentration-effect profiles were obtained by using an indirect model (Figure 1). The following equations were used in calculating the pharmacodynamic parameters:

$$\frac{dR}{dt} = K_{in} - K_{out} \times I \times R \quad \text{(Eq.3)}$$

$$I = \left(1 - \frac{C_p}{(IC_{50} + C_p)}\right) \quad \text{(Eq.4)}$$

$$TT \text{ or } aPTT = R + TT_0 \text{ or } aPTT_0 \quad \text{(Eq.5)}$$

where R is the response (TT or aPTT), K_{in} is the rate constant for production of response, K_{out} is the rate constant for loss of drug response, C_p is the plasma argatroban concentration, and IC_{50} is the drug concentration producing 50% of the maximum inhibition. The pharmacodynamic parameters were calculated by Scientist v. 1.04 (Micromath Scientific Software). The PK/PD model developed at the dose of 0.8 mg/kg was validated by simulating the concentration and effect data at 0.2 and 3.2 mg/kg doses.

Results

Mass spectrometry

Argatroban and the internal standard were eluted at 3.6 and 3.0 min, respectively, with no endogenous or extraneous peaks interfering with the assay. The standard curve was linear over a wide concentration range from 5-10,000 ng/ml (y= 0.000059x -0.000232, r≥0.996). The lower limit of assay quantification was 5 ng/ml using 50 μl of rat plasma. The mean intra- and inter-day assay accuracy ranged from 90.8-110.8% and 89.0-116.9%, respectively, and the mean intra- and inter-day precision was between 4.2-14.9% and 3.5-14.0%, respectively. The extraction recovery was ≥81.4±7.4%, while that of L-375,378 determined at 500 ng/ml was 85.7±3.6%.

Hemolytic Activity of Various Solvents as Dosing Vehicles

The hemolytic activity of various solvents used in intravenous injection is shown in Figure 2. Based on the positive reference, blood mixed with distilled water, DMSO, and mixtures of DMSO and PEG 200 showed relatively high hemolytic activities. As the content of DMSO was reduced in tested solvents, the hemolytic activity was reduced. The DMSO:saline mixture at a ratio of 1:9 showed the lowest

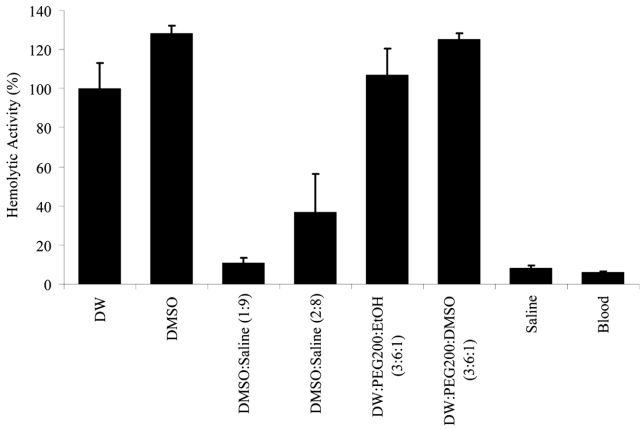

Figure 2–Hemolytic activities of different solvents as dosing vehicles.

hemolytic activity and subsequently used as the dosing vehicle in animal studies.

Intravenous Pharmacokinetics

The drug concentration-time profiles obtained after i.v. injection of argatroban at 0.2, 0.8 and 3.2 mg/kg doses were best described by bi-exponential equations (Figure 3). Compartmental pharmacokinetic parameters are summarized in Table I. The terminal elimination half-life ($t_{1/2}$), systemic clearance (Cl) and steady-state volume of distribution (V_{ss}) ranged from 0.26-0.44 hr, 26.2-54.7 ml/min/kg and 0.27-1.02 l/kg, respectively. There were no significant differences in these parameters as a function of dose. The plasma argatroban concentration extrapolated to time zero (C_0) and the area under the curve (AUC) were linearly increased as the dose was increased.

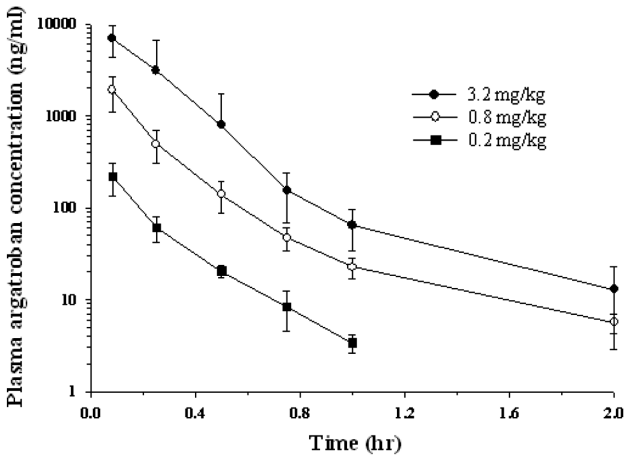

Figure 3–Average plasma argatroban concentration vs. time curves in rats obtained after i.v. injection at 0.2, 0.8, and 3.2 mg/kg doses.

Measurement of TT and aPTT

The mean TT and aPTT data are summarized in Tables II and III, respectively. Values for TT and aPTT were increased as the dose was increased, indicating that the clotting time was prolonged in a dose-dependent manner.

Table I– *Pharmacokinetic parameters of argatroban obtained in rats after i.v. injection at 0.2, 0.8 and 3.2 mg/kg doses (mean±SD)*

Parameters	0.2 mg/kg (n = 5)	0.8 mg/kg (n = 4)	3.2 mg/kg (n = 4)
Weight (kg)	0.30±0.04	0.34±0.02	0.33±0.02
K_{10} (hr^{-1})	7.49± 2.18	8.01±1.62	8.48±4.52
K_{12} (hr^{-1})	2.49±1.90	1.50±0.74	0.87±0.65
K_{21} (hr^{-1})	2.75±1.45	3.72±1.54	3.25±0.74
$t_{1/2}$ (hr^{-1})	0.44±0.28	0.27±0.08	0.26±0.05
C_{max} (ng/ml)	510.2±266.7	4094.6±1983.2	18667.5±12292.3
AUC (ng·hr/ml)	64.9±19.1	509.75±195.1	2356.7±1033.1
Cl (ml/hr)	962.9±179.4	615.7±263.9	524.1±253.4
Cl (ml/min)	16.1±3.0	10.3±4.4	8.7±4.2
Cl (ml/min/kg)	54.7±14.6	29.3±11.1	26.2±11.4
V_{ss} (ml)	288.7±177.6	109.0±44.4	90.0±43.4
V_{ss} (l)	0.29±0.18	0.11±0.04	0.09±0.04
V_{ss} (l/kg)	1.02±0.70	0.31±0.11	0.27±0.12
V_c (ml)	144.8 ± 78.1	78.7 ± 32.2	73.3 ± 37.3
V_p (ml)	132.4 ± 63.8	193.9 ± 72.6	82.4 ± 39.7
AUMC (ng·hr^2/ml)	16.9 ± 5.2	90.8 ± 35.4	495.9 ± 486.7
MRT (hr)	0.29 ± 0.14	0.18 ± 0.02	0.19 ± 0.10

Table II–*Average thrombin time (sec) in rat plasma determined after i.v. injection of argatroban at 0.2, 0.8 and 3.2 mg/kg doses (mean ± SD)*

Time (hr)	0.2 mg/kg (n = 5)	0.8 mg/kg (n = 4)	3.2 mg/kg (n = 4)
0	18.6 ± 5.1	16.5 ± 1.5	20.7 ± 6.9
0.083	53.4 ± 23.9	127.6 ± 55.2	379.8 ± 139.7
0.25	46.4 ± 18.7	48.9 ± 16.1	175.2 ± 54.7
0.5	22.3 ± 5.2	26.3 ± 5.7	67.9 ± 45.2
0.75	19.0 ± 3.4	26.2 ± 8.4	37.1 ± 16.0
1	22.4 ± 8.2	26.2 ± 12.1	28.8 ± 13.1
2	19.2 ± 4.8	21.8 ± 3.7	24.5 ± 6.1
4	19.6 ± 5.2	23.6 ± 5.9	25.1 ± 9.6
6	17.9 ± 2.3	21.0 ± 6.6	19.9 ± 2.4

Table III – Average activated partial thromboplastin time (sec) determined in rat plasma after i.v. injection of argatroban at 0.2, 0.8 and 3.2 mg/kg doses (mean±SD)

Time(hr)	0.2 mg/kg (n =5)	0.8 mg/kg (n = 4)	3.2 mg/kg (n = 4)
0	30.2 ± 0.1	35.6 ± 8.0	33.0 ± 6.2
0.083	45.0 ± 6.4	138.1 ± 25.6	271.0 ± 27.6
0.25	42.4 ± 23.2	62.6 ± 26.5	126.2 ± 50.4
0.5	27.6 ± 0.4	45.0 ± 25.9	95.0 ± 90.0
0.75	33.3 ± 12.4	28.7 ± 9.8	62.7 ± 11.7
1	24.7 ± 2.1	32.6 ± 13.0	61.8 ± 43.9
2	28.3 ± 3.3	32.7 ± 6.5	48.0 ± 27.0
4	25.1 ± 6.6	20.2 ± 3.7	36.5 ± 23.7
6	21.5 ± 1.9	26.1 ± 8.1	19.8 ± 3.3

Table IV – Pharmacodynamic parameters of thrombin time and activated partial thromboplastin time of argatroban in rat derived at a dose of 0.8 mg/kg

Parameters	Thrombin time (TT)	Activated partial t hromboplastin time (aPTT)
K_{in} (hr^{-1})	1.79	0.48
K_{out} (hr^{-1})	0.22	48.3
IC_{50} (ng/ml)	141.4	0.19

Application of PK/PD Model

The pharmacodynamic parameters were derived for TT and aPTT at a dose of 0.8 mg/kg (Table IV). The K_{in}, K_{out} and IC_{50} values were 1.79 hr^{-1}, 0.22 hr^{-1} and 141.4 ng/ml, respectively, for TT, and 0.48 hr^{-1}, 48.3 hr^{-1} and 0.19 ng/ml, respectively, for aPTT. With these values, TT and aPTT were predicted for other doses and compared with the observed data. The predicted and observed data were well correlated (Figure 4).

Discussion

A rapid and sensitive LC/MS/MS assay method was developed for the determination of argatroban in rat plasma. The assay showed a wide linear dynamic range of 5-10,000 ng/mL, with excellent intra- and inter-day accuracy and precision. This assay method was successfully applied to i.v. injection studies of argatroban in rats.

The determination of blood cell lysis was initially reported by Husa et al.[7] This method uses large volumes of the organic water-miscible solvent diluted with saline mixed with small amount of blood at a ratio of 99:1. With this method, the hemolytic activity was 40% for DMSO, 25% for PEG 200, 32% for PG, and 10% for ethanol as they were diluted by saline. Reed et al.[8] and Stenz et al.[6] modified this previous method by mixing the solvent with blood at a ratio of 1:1 or 1:9. With these modified methods, the hemolytic activities were 5.1% for DMSO, 9.7% for PEG 200, 5.7% for PG, and 21.2% for ethanol in saline. The dosing vehicle used in the

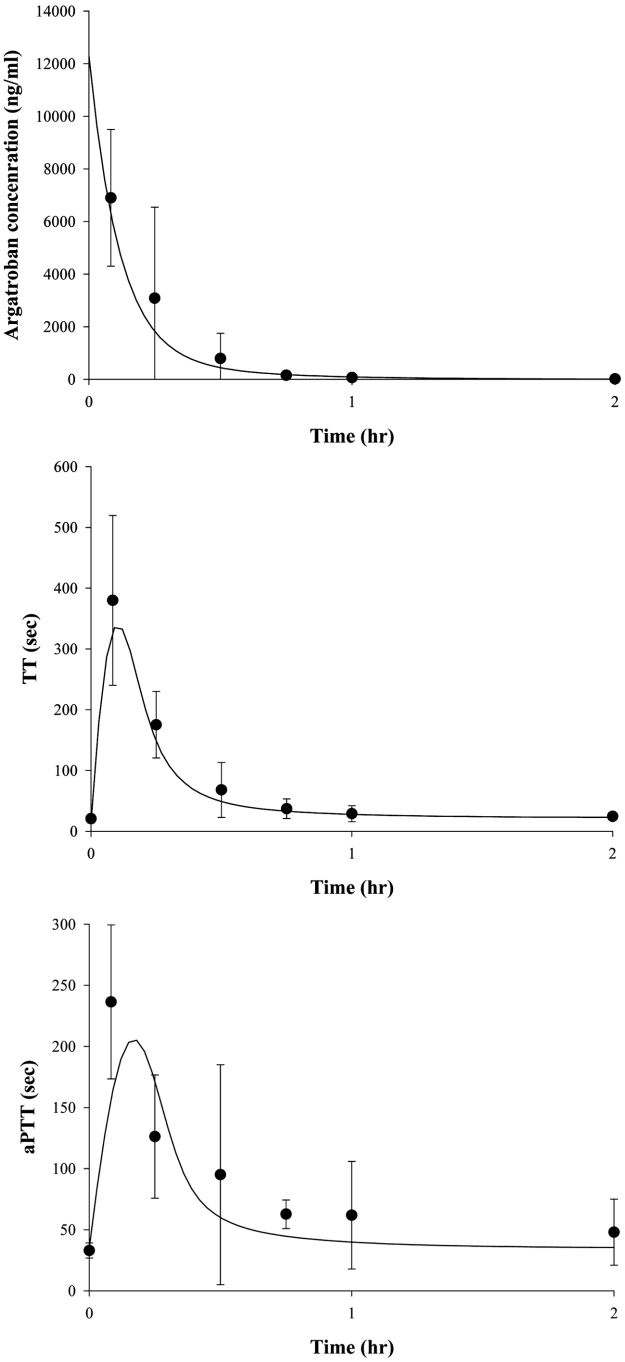

Figure 4–Predicted (solid line) and observed (●) concentration-time profile (upper panel), thrombin time (middle panel) and activated partial thromboplastin time (lower panel) in rat plasma after i.v. injection at 3.2 mg/kg doses.

present PK/PD modeling study was a mixture of DMSO and saline at a ratio of 1:9. It showed a low hemolytic activity (10%) compared to that of blood as the negative reference. This result was not consistent with the hemolytic activity reported by Mottu et al.[9] But, compared with blood and saline, the mixture of DMSO and saline at 1:9 was concluded to have a sufficiently low hemolytic activity to use as the dosing vehicle in the PK/PD study.

Pharmacokinetics and Pharmacodynamics of Argatroban

Argatroban was rapidly eliminated, with $t_{1/2}$ being 0.26-0.44 hr in rats. A blood flow-limited hepatic extraction is suggested by the measured systemic clearance of argatroban of 26.2-54.7 ml/min/kg, based on 60 ml/min/kg of rat liver blood flow.[10] This suggestion was supported with the study of argatroban in human subjects of normal and impaired liver functions.[4] The pharmacokinetics of argatroban was linear over the i.v. dose range of 0.2-3.2 mg/kg. This was evidenced by the observations that Cl and V_{ss} were unaltered, and AUC and C_0 were linearly increased as a function of dose.

Thrombin time is used in the diagnosis of bleeding disorders and the evaluation of anticoagulant therapy. It measures the time when the fibrinogens becomes a fibrin clots. The baseline of thrombin time is known as 10-15 sec. Thrombin time has also been used to evaluate the anticoagulant effects of argatroban[11-13] and was found to be the most sensitive parameter.[14] In this study, the thrombin time was chosen to be one of the markers for the pharmacodynamic study. The peak effect of argatroban in thrombin time was shown at 5 min after i.v. bolus injection, indicating a fast anticoagulation action. The thrombin time returned to the baseline 30 min after drug administration at all 3 doses, which again indicates that argatroban is a fast acting drug with a reversible action. The peak thrombin time was linearly increased as the dose was increased, i.e., it was 53.4±23.9, 127.6±55.2, and 379.8±139.7 sec at 0.2, 0.8, and 3.2 mg/kg doses, respectively.

Activated partial thromboplastin time (aPTT) is commonly used to assess the function of the intrinsic pathway in the blood coagulation pathway. It measures most sensitively the functional deficiencies of factor VII, IX, XI, XII, prekalikrein and high-molecular-weight kininogen. The baseline of aPTT is known as 25 to 35 sec. Studies of aPTT with various anticoagulants have been performed, including heparin[4] and direct thrombin inhibitors such as efegatran, hirulog and hirudin[15] and argatroban.[2,11] In the present study, aPTT was chosen to be an additional pharmacodynamic marker. The baseline was the aPTT measured prior to drug administration, which was in the range of reported baselines. The peak effect of argatroban in aPTT was shown at 5 min after i.v. injection, again indicating a fast anticoagulating action. The aPTT returned to the baseline 30 min after drug administration at 0.2 and 0.8 mg/kg doses but was prolonged for 2 hr at the highest dose (3.2 mg/kg). The peak aPTT was linearly increased as the dose was increased.

Application of PK/PD Model

In PK/PD modeling, both pharmacokinetic and pharmacodynamic parameters are needed to link the dose-concentration-effect relationship. Of 3 major pharmacokinetic models of compartmental, flow and statistical models, the compartmental models are frequently used in the PK/PD modeling as they provide a continuous drug concentration at the effect site. In this study, a 2-compartment model was used as the i.v. pharmacokinetics of argatroban was best described by bi-exponential equations. In the reversible pharmcodynamic effects of drugs, either direct or indirect response model is used. The indirect model is used for drugs that exhibit a lag time in the response that is not directly related with the drug concentration at the site of action. The anticoagulant effect of warfarin has been described by the indirect model.[16] In the present study, the indirect model was used in the assessment of the pharmacodynamics of argatroban due to the mechanism of inhibiting thrombin prior to exerting an anticoagulant effect. Among different indirect response models, k_{out} inhibition model best fit the pharmacodynamics of argatroban. In this study, TT and aPTT were chosen to estimate the pharmacodynamic outcomes. Using the PK/PD model developed at a dose of 0.8 mg/kg, the concentration-time profiles and pharmacodynamic effects were simulated and validated at other doses. The observed and simulated profiles agreed well, indicating that an appropriate model selection was made.

Conclusions

A PK/PD model with an indirect k_{out} linkage was developed to describe the concentration-effect relationship of argatroban. The model was validated by comparing the simulated and observed concentration-effect profiles. The observed concentration-time profiles and the pharmacodynamic (TT and aPTT) profiles agreed well with the simulated profiles, indicating that an appropriate PK/PD model was utilized.

References

1) L.R. Bush, Argatroban, a selective, potent thrombin inhibitor, *Cardiovas. Drug Rev.*, **9**, 247-263 (1991).
2) T. Tamao, T. Yamamoto, T. Hirata, M. Kinusaga and R. Kiku-

moto, Effect of argatroban on blood coagulation, *Jap. J. Pharm. Ther.*, **14**, 7-12 (1986).
3) K. McKeage and G.L. Plosker, Argatroban, *Drugs,* **61**, 515-522 (2001).
4) S.K. Swan and M.J. Hursting, The pharmacokinetics and pharmacodynamics of argatroban: effects of age, gender, and hepatic or renal dysfunction, *Pharmacotherapy,* **20**, 318-329 (2000).
5) S. Ahmad, A. Ahsan, M. George, O. Iqbal, W.P. Jeske, R. McKenna, B.E. Lewis, J.M. Walenga and J. Fareed, Simultaneous monitoring of argatroban and its major metabolite using an HPLC method: potential clinical applications, *Clin. Appl. Thromb. Hemost.,* **5**, 252-258 (1999).
6) R. Stenz and K.H. Bauer, A new physiologically approached in vitro test for quick evaluation of the hemolytic activity of surfactants, *Pharmazie,* **51**, 283-287 (1996).
7) W.J. Husa and J.R. Adams, Isotonic solutions. II. The permeability of red corpuscles to various substances, *J. Am. Pharm. Assoc. Sci. Ed.,* **33**, 329 (1944).
8) K.W. Reed and S.H. Yalkowsky, Lysis of human red blood cells in the presence of various cosolvents, *J. Parenter. Sci. Technol.,* **39**, 64-69 (1985).
9) F. Mottu, A. Laurent, D.A. Rufenacht and E. Doelker, Organic solvents for pharmaceutical parenterals and embolic liquids, *PDA J. Pharm. Sci. Technol.,* **54**, 456-469 (2000).
10) J. Hauptmann, Pharmacokinetics of an emerging new class of anticoagulant/antithrombotic drugs. A review of small-molecule thrombin inhibitors, *Eur. J. Clin. Pharmacol.,* **57**, 751-758 (2002).
11) S. Ahmad, L.H. Yang, A. Ahsan, K. Fu, O. Iqbal, D.A. Hoppensteadt, B.E. Lewis, J.M. Walenga and J. Fareed, Pharmacokinetics of argatroban in primates: evidence on endogenous uptake, *Int. Angiol.*. **19**, 126-34 (2000).
12) C.N. Berry, C. Lunven, C. Lecoffre, P. Lainee, S.E. O'Connor, F. Andre, B. Roger, D. Garrigou-Gadenne, A. Rouchouse, N.O. Roome and N. Vivan, Anticoagulant activity and pharmacokinetic properties of a sub-cutaneously administered mixed micellar formulation of argatroban in experimental animals, *Thromb. Haemost.*, **84**, 278-285 (2000).
13) N. Duval, C. Lunven, D.P. O'Brien, A. Grosset, S.E. O'Connor and C.N. Berry, Antithrombotic actions of the thrombin inhibitor, argatroban, in a canine model of coronary cyclic flow: comparison with heparin, *Br. J. Pharmacol.*, **118**, 727-733 (1996).
14) W. Jeske, J.M. Walenga, B.E. Lewis and J. Fareed, Pharmacology of argatroban, *Expert Opin. Invest. Drugs,* **8**, 625-654 (1999).
15) D. Callas and J. Fareed, Comparative pharmacology of site directed antithrombin agents. Implication in drug development, *Thromb. Haemost.*, **74**, 473-481 (1995).
16) R. Nagashima, R.A. O'Reilly and G. Levy, Kinetics of pharmacologic effects in man: the anticoagulant action of warfarin, *Clin. Pharmacol. Ther.*, **10**, 22-35 (1969).