J. Pharm. Pharmacol. 1996, 48: 573–577
Received June 27, 1995
Accepted December 5, 1995

Animal Pharmacokinetics and Interspecies Scaling from Animals to Man of Lamifiban, a New Platelet Aggregation Inhibitor

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Abstract

Relating pharmacokinetic information obtained in animal species to man (interspecies scaling) can play an important role in enabling understanding of the differences and similarities between species, and helping to predict the kinetic profile of a new compound in man.

Interspecies scaling techniques have been applied to lamifiban (Ro 44-9883), a new selective and potent non-peptidic inhibitor of human glycoprotein IIb-IIIa intended for use in clinical treatment of, for example, acute coronary syndrome. The pharmacokinetic profile of lamifiban in man was predicted from animal data (in rats, dogs and cynomolgus monkeys) by using allometric scaling and concentration–time transformations. These extrapolations for lamifiban were performed prospectively, to help design the first pharmacokinetic studies in man. The approach based on equivalent time was preferred for our prospective predictions, in view of the high values found for the allometric exponents. Using allometric scaling, clearance (CL), half-life($t_{1/2}$) and volume of distribution (Vd) were overestimated by approximately two- to fourfold. Compared with allometric scaling, the transformation based on equivalent time improved the prediction for all human pharmacokinetic parameters. For $t_{1/2}$ and CL, the observed values for man were within the range predicted from the various animal species.

Of the individual animal species, the cynomolgus monkey gave the most reliable predictions of these two parameters, as well as accurately predicting the Vd value.

Relating the pharmacokinetic information obtained in animal species to man is an important issue in drug development because interspecies scaling can play an important role in providing understanding of the differences and similarities between species, and in predicting the kinetic profile of a new compound in man (Lave et al 1995). Several reports dealing with interspecies scaling have recently been published. Allometric scaling has been successfully applied to various drugs, for example small organic molecules such as antibiotics (Swabb & Bonner 1983; Sawada et al 1984; Mordenti 1986; Efthymiopoulos et al 1991) and to proteins (Mordenti et al 1991). In this study, interspecies scaling techniques were applied to lamifiban (Ro 44-9883), a new selective and potent non-peptidic inhibitor of human glycoprotein IIb-IIIa (GP IIb-IIIa) which is intended for use in clinical treatment of, for example, acute coronary syndrome. GP IIb-IIIa is the platelet receptor for the adhesive proteins fibrinogen, fibronectin and von Willebrand factor (Plow & Giusberg 1988). All platelet agonists, including thrombin, lead to the activation of this receptor, resulting in aggregation and thrombus formation by cross-linking of the platelets. Selective inhibition of GP IIb-IIIa therefore represents the most powerful mechanism for inhibition of platelet aggregation (Coller 1990).

The pharmacokinetics of lamifiban were assessed after intravenous administration to rats, dogs and cynomolgus monkeys. The profile in man was then predicted from these animal data by using allometric scaling, where a given pharmacokinetic parameter (P) is related to body weight (B) according to a power function $P = aB^b$. An alternative interspecies scaling technique, based on concentration-time transformations (Dedrick et al 1970; Boxenbaum & Ronfeld 1983), was also used to estimate the kinetic profile in man. These extrapolations for lamifiban were performed prospectively, to help design the first pharmacokinetic studies in man. The values estimated by allometric scaling and the concentration-time transformations were then compared with those observed in the first clinical trial.

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Materials and Methods

Animal pharmacokinetics

Male rats (250–300 g; SPF, RoRo, BRL, Füllinsdorf, Switzerland) were injected intravenously with a single dose of lamifiban (2·2 mg kg^{-1}, n = 2; 22 mg kg^{-1}, n = 4). The test compound was administered through a catheter implanted into the jugular vein, and blood samples were withdrawn after 3, 15 and 30 min, and 1, 1·5, 1·75, 2, 2·25, 2·5, 3, 4·5 and 6 h.

Male beagle dogs (14–16 kg) were injected intravenously with an infusion of lamifiban (28 $\mu g\ kg^{-1}\ min^{-1}$, n = 2; 3·3 $\mu g\ kg^{-1}\ min^{-1}$, n = 2) for 2 h. Blood samples were collected by venipuncture 30 min, and 1, 1·5, 2, 2·05, 2·25, 2·5, 3, 4, 5, 6, 7, 8, 9, 10, 24 and 27 h after starting the infusion.

Cynomolgus monkeys (*Macaca fasicularis*), 2·5–3·6 kg (n = 4), received lamifiban (20 $\mu g\ kg^{-1}\ min^{-1}$) as an intravenous infusion for 30 min. Blood samples were obtained from a brachial vein catheter 10, 20, 30, 40 and 50 min, and 1, 1·5, 2·5, 3·5, 4·5 and 6·5 h after starting the infusion.

Lamifiban plasma concentrations were determined in the various species by means of a specific HPLC-UV method. In summary, lamifiban was extracted from plasma using octyl solid-phase extraction cartridges and separation was performed by reversed-phase HPLC with gradient elution. Detection was by UV absorption at 244 nm. The limit of quantification of the assay was 25 ng mL^{-1}.

Pharmacokinetic analysis

Plasma concentration vs time data in each species were analysed by non-compartmental methods. The area under the curve (AUC) and the area under the moment curve (AUMC) were calculated using the logarithmic trapezoidal rule, and then extrapolated to time infinity by adding c/β to AUC and $tc/\beta + c/\beta^2$ to AUMC, where c is the last predicted concentration at the last sampling time t. For extrapolation of the trapezoidal area under the data curve from the first data point to time t_0 after administration, the first data point was connected with the origin for an intravenous infusion and a horizontal extrapolation from the first data point to the time t_0 was performed after bolus administration. The slope of the terminal phase, β, was determined by log-linear regression of the last three or four data points, and the terminal half-life ($t_{1/2}$) was calculated from $t_{1/2} = 0.693/\beta$. The systemic clearance (CL) was calculated using the relationship CL = Dose/AUC. The volume of distribution at steady state (Vd_{ss}) was calculated from $Vd_{ss} = CL \times AUMC/AUC$ for intravenous bolus administration and $Vd_{ss} = CL \times (AUMC/AUC - IT/2)$, where IT is the infusion time, for intravenous infusion.

Allometric scaling

For allometric scaling, the mean pharmacokinetic parameters for CL, Vd_{ss} and $t_{1/2}$ of lamifiban in animals were correlated with the body weight (B), using the allometric equations $CL = aB^x$, $Vd_{ss} = bB^y$ and $t_{1/2} = cB^z$, where a, b and c are the allometric coefficients and x, y and z are the allometric exponents. The values of exponents and coefficients were estimated by least squares fitting of log CL vs log B, log $t_{1/2}$ vs log B and log Vd_{ss} vs log B.

Using the allometric equations obtained for CL, $t_{1/2}$ and Vd_{ss}, values corresponding to a 70 kg man were estimated and compared with the data observed in man (Jones et al unpublished data).

Pharmacokinetic time calculations

Pharmacokinetic times were calculated from the chronological time by use of the methods of Dedrick (1970), Boxenbaum & Ronfeld (1983) and Boxenbaum (1984). Chronological time was normalized to 'equivalent time' by dividing the post-injection time by $B^{0.25}$. Chronological time (t) was converted to kallynochrons by dividing by B^{1-x}, where x is the allometric exponent of CL. The calculation of kallynochrons assumes that Vd_{ss} is directly proportional to B. Apolysichrons are defined as t/B^{y-x}, where y and x are the allometric exponents of Vd_{ss} and CL, respectively. Kallynochrons and apolysichrons are equivalent when Vd_{ss} in the different species is directly proportional to B. When using 'equivalent time' or kallynochrons, the plasma concentrations were normalized by the dose administered per kg of body weight. In the case of apolysichrons, plasma concentrations were normalized by dividing by $dose/B^y$. Each animal's time unit (t_{animal}) was converted into human time (t_{human}) by use of the equations 1–3:

when using 'equivalent time':

$$t_{human} = t_{animal} \times (B_{human}/B_{animal})^{0.25} \quad (1)$$

when using kallynochrons:

$$t_{human} = t_{animal} \times (B_{human}/B_{animal})^{1-x} \quad (2)$$

when using apolysichrons:

$$t_{human} = t_{animal} \times (B_{human}/B_{animal})^{y-x} \quad (3)$$

As the data from dog and cynomolgus monkey were obtained after intravenous infusion, the corresponding profiles of plasma concentrations against time for a bolus administration in these two species were simulated before applying the concentration-time transformations. These simulations were based on a two-compartment model, and were performed using Stella (Washington et al 1987).

Results

The mean plasma concentration vs time data obtained from rats, dogs and cynomolgus monkeys after intravenous administration are illustrated in Fig. 1. The elimination half-lives were 0·45 to 2·1 h in rats and dogs, respectively. Lamifiban showed volumes of distribution at steady-state (Vd_{ss}) of 0·24 and 0·29 L kg^{-1} in rats and cynomolgus monkeys, respectively, and 0·75 L kg^{-1} in dogs. The total clearances were 2·6 and 10 mL min^{-1} kg^{-1} in cynomolgus monkeys and rats, respectively. Most of the clearance occurs by excretion of the unchanged compound in bile and urine. Because rats and dogs respectively excreted 60 and 40% of the compound intact in urine, their renal clearances of lamifiban (5·6 and 2·8 mL min^{-1} kg^{-1}, respectively) were smaller than the corresponding glomerular filtration rates (8·7 and 4·5 mL min^{-1} kg^{-1}, respectively (Davies & Morris 1993)), indicating some renal re-absorption.

Transforming these pharmacokinetic parameters in the various animal species to the corresponding values based on total animal gives the data presented in Table 1. These values were then used to generate the allometric equations.

Table 2 shows the results of least squares fitting of log $t_{1/2}$, log CL and log Vd_{ss} vs log B in the animals. Pharmacokinetic parameters corresponding to a 70-kg man were then estimated using the allometric equations obtained from the animal data. The observed parameters in man are also presented in Table 2 for comparison with the predictions. The results in man were obtained after intravenous infusions of lamifiban (0·6 and 1·0 μg kg^{-1} min^{-1}, n = 3 and n = 6, respectively) for 30 min to healthy volunteers.

The regression equations for plots of log $t_{1/2}$, log CL and log Vd_{ss} vs log B, obtained using data in animals, are illustrated in Figs 2–4, together with the mean value subsequently found in man.

The allometric exponents of volume of distribution and clearance in Table 2 were then used to superimpose the plasma

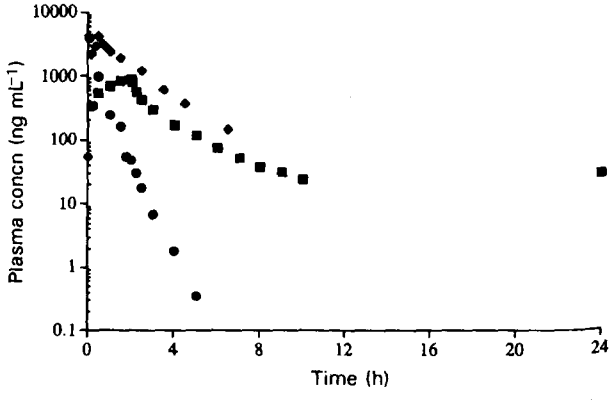

FIG. 1. Profiles of mean plasma concentrations against time for lamifiban in rats (●), dogs (■) and cynomolgus monkeys (◆) after intravenous administration.

Table 1. Mean pharmacokinetic parameters of lamifiban in different animal species after intravenous administration.

Species	Weight (kg)	t½ (h)	CL_{Tot} (mL min^{-1})	CL_{Ren} (mL min^{-1})	Vd_{ss} (L)
Rat (n = 6)	0·275	0·45	2·6	1·5	0·066
Dog (n = 2)	15·0	2·1	105	42	11·25
Monkey (n = 4)	3·2	1·4	8·3	ND	0·928

ND = not determined.

Table 2. Allometric interspecies scaling of the pharmacokinetic parameters of lamifiban in animals and comparison with the estimated parameter values in man.

Pharmacokinetic parameter	Allometric equation	Correlation coefficient (r^2)	Human data	
			Estimated	Observed
t½ (h)	$0·784 \times B^{0·386}$	0·979	4·2	2·10
CL_{Tot} (mL min^{-1})	$6·08 \times B^{0·886}$	0·889	259	134
Vd_{ss} (L)	$0·295 \times B^{1·268}$	0·987	63·7	20·3

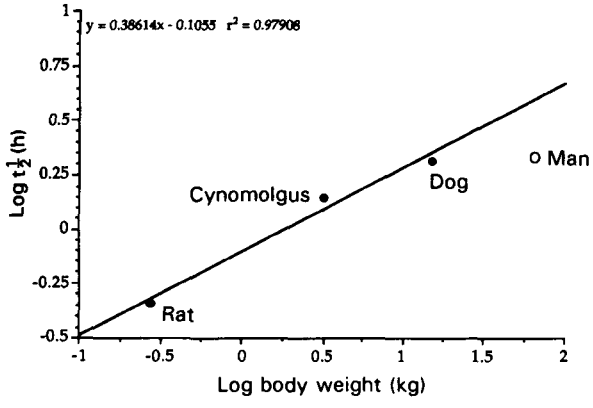

FIG. 2. Allometric scaling of lamifiban half-life (t½).

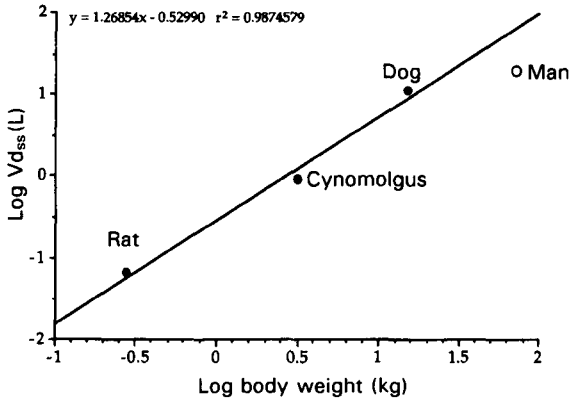

FIG. 4. Allometric scaling of lamifiban volume of distribution (Vd_{ss}).

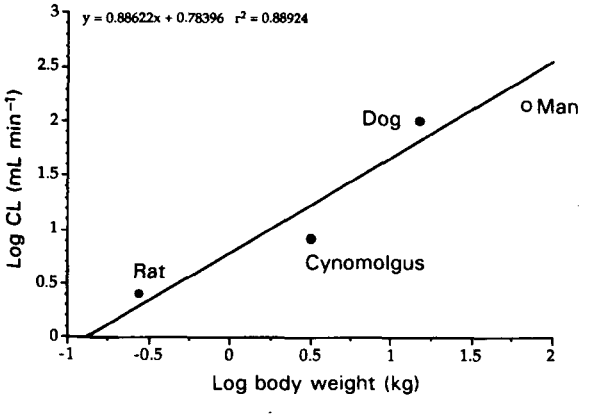

FIG. 3. Allometric scaling of lamifiban clearance (CL).

concentration vs time profiles in the different animal species. The representations using "equivalent tim", apolysichrons and kallynochrons are illustrated in Figs 5–7, respectively. The corresponding pharmacokinetic parameters are presented in Table 3.

Discussion

The pharmacokinetic parameters of lamifiban correlate well with body weight using the allometric approach, as previously described for other small organic molecules. This is because its pharmacokinetics fulfil the main criteria for allometric scaling – first order kinetics and elimination by physical transport processes. Direct evidence that the kinetics of lamifiban were first order was obtained in rats and dogs – the parameters were independent of dose in the ranges: 2–20 mg kg^{-1} in rats and 0·3–3·5 mg kg^{-1} in dogs. Lamifiban is, furthermore, very weakly bound to plasma proteins (6% in man, 11% in dogs and 8% in rats), indicating that protein binding is not important for interspecies scaling of this test compound.

The allometric exponents obtained for the half-life (h), clearance (mL min^{-1}) and volume of distribution (L) of lamifiban were 0·386, 0·886 and 1·268, respectively. The exponent for half-life is close to the upper limit of values expected for physiological processes and for small organic molecules (0·2 to 0·4 (Mordenti 1986)). Similarly, the exponents for volume of distribution and clearance are slightly larger than those reported for other molecules (0·8–1·0 for Vd and 0·6–0·8 for CL). For both parameters this is because of the

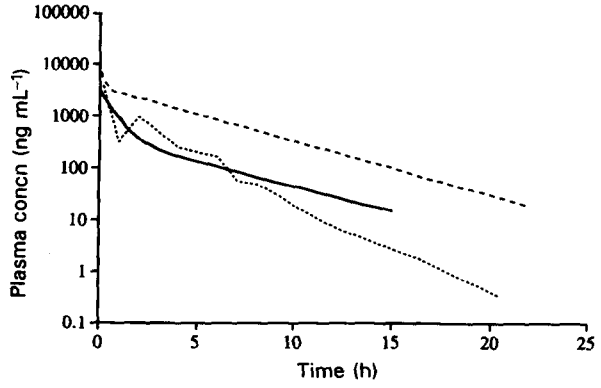

FIG. 5. Concentration–time profile of lamifiban predicted in man by using equivalent time. Dog —, cynomolgus monkey ---, rat ···.

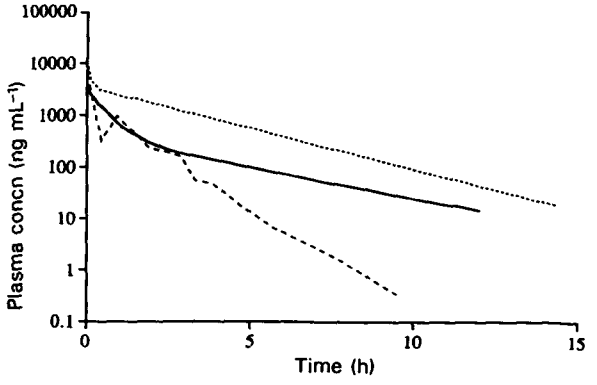

FIG. 7. Concentration–time profile of lamifiban predicted in human by using kallynochrons. Dog —, cynomolgus monkey ---, rat ···.

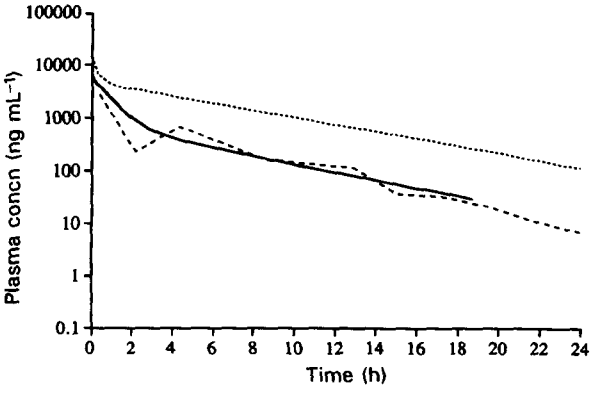

FIG. 6. Concentration–time profile of lamifiban predicted in man by using apolysichrons. Dog —, cynomolgus monkey ···, rat ---.

important influence of the dog on the slope of the regression line, especially for volume of distribution, where the value in the dog (0·75 L kg^{-1}) is high in comparison to those in the rat and cynomolgus monkey (0·24 and 0·29 L kg^{-1}, respectively).

To predict the human results prospectively, the weight of a healthy volunteer (70 kg) was substituted into the allometric equations for the various pharmacokinetic parameters. Compared with the observed values, clearance and half-life were overestimated by approximately twofold. Because of the high value observed in dogs, the volume of distribution was, furthermore, overestimated by more than fourfold. In view of the high values found for the allometric exponents, these overestimates of the human kinetic parameters were not unexpected.

Representations based on the various concentration–time plots were then attempted and compared with direct allometric scaling. Using a fixed exponent for body weight (i.e. 0·25) to transform chronological time in the different species into equivalent time was a successful approach for lamifiban. In contrast with data based on kallynochrons and apolysichrons, the advantage of this approach is, furthermore, that prior estimation of the allometric exponents for clearance and volume of distribution is not necessary. The concentration-time transformations based on 'equivalent time' are, therefore, not influenced by the accuracy of the predictions from allometric scaling. For t½ and CL, the observed mean values of 2·1 h and 134 mL min^{-1} for man were within the range predicted from the various animal species (1·7–3·2 h and 75·3–346 mL min^{-1} for t½ and CL, respectively). Of the individual animal species, the cynomolgus monkey gave the most reliable predictions of these two parameters, as well as an accurate prediction of the volume of distribution (18·1 L compared with 20·3 L observed). Compared with allometric scaling, the transformation based on equivalent time improved the prediction of all the human pharmacokinetic parameters.

With apolysichrons, as with allometric scaling, the t½ observed in man was overestimated by a factor of two. This was expected because the data from apolysichrons include components which are derived from the direct allometric scaling of clearance and volume of distribution which also lead to twofold overestimation of half-life. The ranges predicted for CL and Vd_{ss} were, furthermore, larger than those obtained using equivalent time.

The estimates based on kallynochrons were similar to the estimates obtained using equivalent time. Because the calculation of kallynochrons and equivalent time assume that volume of distribution is directly proportional to B, identical estimates for Vd_{ss} were obtained with these two transformations.

In man, lamifiban is excreted mainly (ca 90%) unchanged in

Table 3. Predicted pharmacokinetic parameters of lamifiban in man according to plasma concentration extrapolation methods.

Parameter	Equivalent time	Apolysichrons	Kallynochrons	Observed
t½ (h)	3·2/1·7/2·9	4·0/3·8/4·4	2·5/0·79/1·9	2·10
CL_{Tot} (mL min^{-1})	346·2/248·4/75·3	171·4/352·2/45·1	429·0/526·8/120·4	134
Vd_{ss} (L)	53·7/26·9/18·1	38·7/112·9/17·3	53·7/26·9/18·1	20·3

Data prediction from dog/rat/monkey, respectively; underlined values are closest to the values observed for man.

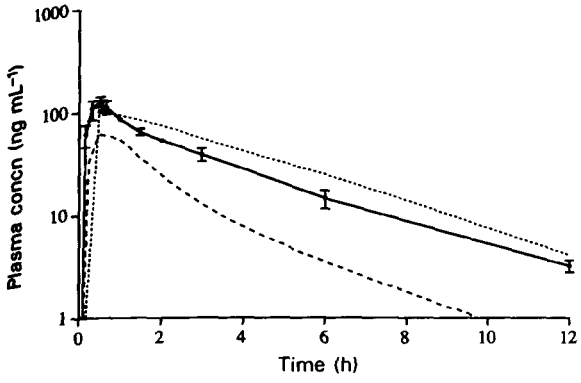

FIG. 8. Comparison of predicted and observed concentration–time profiles of lamifiban in man. The predicted profile was based on equivalent time transformation. Mean (±s.d.) plasma concentrations were obtained from healthy subjects (n=6) following intravenous infusion of lamifiban (1 $\mu g\ kg^{-1}\ min^{-1}$ for 30 min). Predicted from rat ---, predicted from cynomolgus monkey · · ·, data observed in man —o—.

urine, indicating differences in the excretion pathways compared with rat and dog, where biliary excretion of the unchanged compound represents 40 and 60%, respectively, of the dose administered. This observation agrees with the interspecies differences in molecular weight threshold for biliary excretion: the molecular weight of lamifiban (468) is higher than the biliary excretion threshold in rat and dog (300) which are good biliary excretors, but lower than the corresponding threshold in man and monkey (500–600), which are poor biliary excretors (Fleck & Bräunlich 1990). Thus, extrapolating renal clearance from rat and dog to man predicts a value of 158 mL min^{-1}, which is very close to the total clearance observed in man (134 mL min^{-1}). Also, although urinary and biliary data are not available for the cynomolgus monkey, because this species accurately predicted the human pharmacokinetic parameters of lamifiban, it can be assumed that the excretion mechanisms in monkey and in man are very similar.

These kinetic extrapolations of lamifiban from animals to man were performed before initiation of the first clinical trial. The disposition in man after various dosage regimens was, therefore, predicted from interspecies scaling (Fig. 8). The approach based on equivalent time was preferred for our prospective predictions, in view of the high values found for the allometric exponents. Despite the interspecies differences in clearance mechanisms, the kinetic profile of lamifiban in man was predicted successfully, and the information obtained from the animals resulted in a more efficient design of the first clinical studies.

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