Pharmacokinetics and Pharmacodynamics of L-703,014, a Potent Fibrinogen Receptor Antagonist, After Intravenous and Oral Administration in the Dog

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The pharmacokinetics and pharmacodynamics of L-703,014, a fibrinogen receptor antagonist, have been examined in the dog. An analytical method which utilizes methanol precipitation of dog plasma proteins followed by HPLC with an automated column switching technique using the chemical analogue L-704,326 as internal standard was developed for the determination of L-703,014 in dog plasma. The compound was not metabolized in the dog and was eliminated in the kidneys and into bile. Of the administered dose, 68.9 ± 1.3% (i.v.) and 80.5 ± 11.9% (p.o.) were recovered in the feces; 20.3 ± 1.3% (i.v.) and 2.2 ± 0.2% (p.o.) were recovered in the urine by 72 hr. L-703,014 was 23 ± 3.4% bound in dog plasma protein and the mean ratio of plasma/whole blood was 1.22 ± 0.05. The mean terminal half-life was 118 ± 36 min, the mean steady-state volume of distribution was 0.61 ± 0.22 L/kg, and the mean plasma clearance was 8 ± 2 mL/min/kg. *Ex vivo* platelet aggregation measurements were made by inducing platelet aggregation with 10 μg/mL collagen in the presence of 1 μ*M* epinephrine as an agonist. The mean C_{50} was 44.4 ± 6.0 ng/mL, and the mean Hill coefficient was 1.5 ± 0.3. The mean bioavailability was 4.9 ± 1.4% in dogs administered 2.0 mg/kg (p.o.).

KEY WORDS: fibrinogen receptor antagonist; pharmacokinetics; pharmacodynamics.

INTRODUCTION

Pharmacologic antagonism of platelet activation and aggregation is considered a viable therapeutic strategy for patients suffering from acute arterial occlusions, myocardial infarction, stroke, and a variety of other vascular disorders. Blocking the binding of fibrinogen to the GP IIb/IIIa receptor complex is one mechanism by which several compounds under investigation act as intravenous antiplatelet agents (1,2). The use of pharmacokinetics and pharmacodynamics in understanding the basic pharmacology of antiplatelet drugs and their benefit in the selection of optimal dose regimens has been emphasized in the literature (3) since antiplatelet drugs may reversibly or irreversibly inhibit platelet aggregation. Additionally, it has recently been theorized (4) that the elimination rate constant of the drug in question and the dissociation rate constant of the ligand–receptor complex can influence the pharmacodynamic time course of such agents. L-703,014 is a novel nonpeptide antagonist of GP IIb/IIIa and has nanomolar potency and a high degree of selectivity for GP IIb/IIIa over other integrins (5). This report describes the pharmacokinetic and pharmacodynamic characteristics of L-703,014 in the dog.

METHODS

In Vitro Studies

The partition of ^{3}H-L-703,014 between plasma and the red blood cell fraction was studied by incubation of the drug with whole blood from the dog at 37°C. Nonlabeled L-703,014 concentration and incubation time were examined and did not affect the partitioning results. Plasma protein binding was examined over the range of 0.01 to 25 μg/mL of L-703,014 in dog plasma. Nonspecific binding of the drug was also determined for all concentrations.

The *in vitro* IC_{50} was determined as described previously (6). The specificity for platelet aggregation was examined by assessing the effect of L-703,014 on human endothelial cell attachment to adhesive proteins. Microtiter plates were coated with 0.1 mL/well of 5 n*M* human plasma fibrinogen (Calbiochem), 8 n*M* human plasma vitronectin (Calbiochem), 3.2 n*M* human plasma fibronectin (Calbiochem), or 2% bovine serum albumin (BSA; control). After coating, plates were blocked with 2% BSA. Five thousand endothelial cells were added, with or without L-703,014, to each well. Plates were covered and incubated at 37°C, 2.2% CO_2, for 75 min. Times and coating concentration of proteins were selected in preliminary experiments. Following washing, adherent cells were removed by trypsin-EDTA and transferred to vials containing PCS scintillation fluid (Amersham). Radioactivity was measured in a scintillation counter. Percentage inhibition values for each dilution were calculated.

Analytical

L-703,014 and L-704-326 (internal standard; see Fig. 1 for structures) were obtained from the Department of Medicinal Chemistry, Merck Research Laboratories. HPLC with automated column switching and fluorescence detection was performed with two Model M-510 high-pressure pumps, a Model 712 WISP autosampler, a Model 840 HPLC system controller and data system (Waters Associates, Milford, MA), and a Jasco Model 820-FP (Jasco, Inc., Easton, MD) or Waters Model 470 fluorescence detector. An Autochrom Model 201 solenoid interface, Autochrom Model 7001 automated solvent switching valve, and Autochrom Model 401A pneumatically actuated switching valve (Thom-

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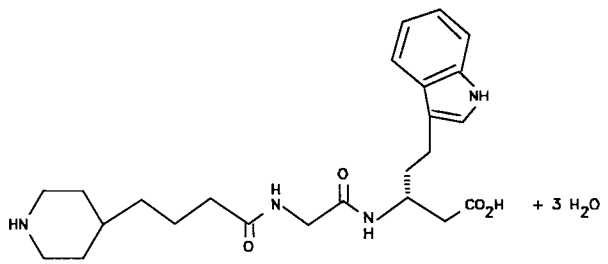
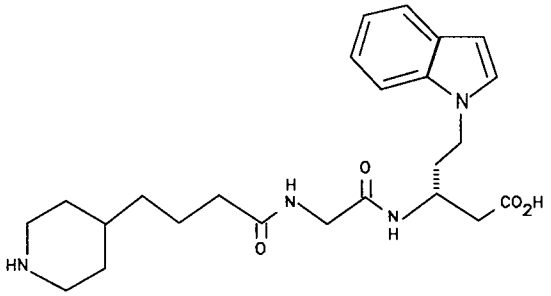
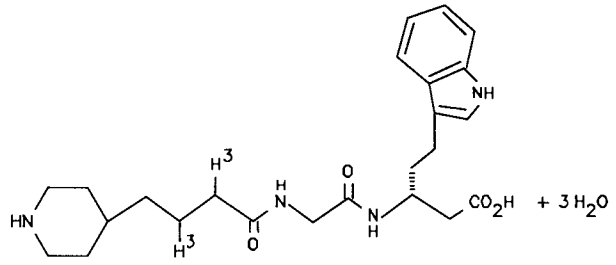


L-703,014



L-704,326 (Internal Standard)



^{3}H-L-703,014

Fig. 1. Chemical structures for L-703,014, ^{3}H-L-703,014, and L-704,326 (internal standard).

son Instruments, Newark, DE) were used for the automated column switching sample cleanup technique.

A Bio-Rad CN-guard column cartridge (30 × 4.6 mm) served as the concentration column for sample cleanup and a Waters Novapak C-18 column (150 × 3.9 mm) was used for chromatographic separation of drug and internal standard. The sample extract was injected onto the concentration column with a mobile phase of 0.1% ammonium bicarbonate, pH 7.0, at 1.0 mL/min (sample on/flush precolumn mode). After a 6-min wash of the concentration column, the drug and internal standard were backflushed off the concentration column and switched onto the analytical column with 10% CH_3CN/0.1% ammonium bicarbonate, pH 7.8, buffer at a flow rate of 1.0 mL/min (elution mode). After a 3-min elution of the concentration column (6–9 min), the valve was switched back to the sample on/flush mode and the concentration column was then washed with 60% CH_3CN/0.1% ammonium bicarbonate, pH 7.0, buffer at a flow rate of 1.0 mL/min from 9.1 to 17 min at the same time that drug and internal standard were being chromatographed on the analytical column. The concentration column wash was accomplished by timed event contact closure switches to the solvent selection valve on pump A to elute contaminants from the concentration column in preparation for the next injection (9.1–17 min). At 17 min, pump A was then switched back to the sample on mobile phase to reequilibrate the concentration column for the next injection.

Detection of L-703,014 and internal standard was achieved by fluorescence with excitation and emission wavelengths at 280 and 365 nm, respectively. Gain, attenuation, emission bandpass, and filtering on the detector were set to ×1000, ×1, 30 nm, and slow, respectively. Drug and internal standard were added to plasma as aqueous solutions in volumes that did not exceed 5% of total plasma volume. After the addition of drug and internal standard, 5 mL of methanol was added to precipitate plasma proteins. The samples were then centrifuged at 2000*g* for 10 minutes. The supernatant was decanted into clean test tubes and evaporated to dryness under N_2 at room temperature. The dry residue was reconstituted in 0.20 mL of 0.1% ammonium bicarbonate (pH 7.8) and filtered through Acrodiscs (0.45 μm, PVDF, Model LC-13, Gelman Sciences, Ann Arbor, MI) into autosampler vials for HPLC analysis. From 10 to 50 μL (4–25%) of the extract was injected on column for quantitation of L-703,014. Extraction efficiency was 92 ± 2% at 2 ng/0.2 mL under these conditions as determined by assay with radioactive drug. The method was validated from 1 to 400 ng/mL. The intraday coefficients of variation ranged from 0.4 to 7.5% for L-703,014. The interday coefficients of variation for high, medium, and low controls ranged from 1.2 to 10.7%.

Administration

Four mongrel dogs were included in a pharmacokinetic/pharmacodynamic (PK/PD) oral–i.v. crossover study (AN Nos. 1–4) and four other dogs were used in the oral–i.v. excretion and metabolism studies (AN Nos. 5–8). ^{3}H-L-703,014 (3.4 μCi/mg) and nonlabeled L-703,014 were dissolved in 0.9% saline (5 mL) and administered intravenously to mongrel dogs via the cephalic vein as a bolus dose of 0.2 mg/kg. After a 1-week washout, the dogs received ^{3}H-L-703,014 or nonlabeled L-703,014 in a water solution orally by gavage at a dose of 2.0 mg/kg (5 mL with 10 mL water flush). Blood for L-703,014 plasma concentration determination was drawn at predose and 2, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300, and 360 min after i.v. administration and at predose and 20, 30, 40, 50, 70, 90, 110, 150, 200, 250, 300, 350, and 480 min after oral administration. Blood (7 mL) was collected into 13 × 100-mm glass tubes or polypropylene microtubes containing heparin as anticoagulant. Immediately after collection, blood was centrifuged at 2000*g* for 7 min to separate the plasma, which was used fresh or frozen at −70°C for later use.

Animals were housed in individual metabolism cages. Urine and feces were collected for 72 hr after dosing. Urine was collected over dry ice and stored at −20°C until assay for radioactivity, ^{3}H-L-703,014, and metabolites. Feces were homogenized in approximately 5 volumes of H_2O with a Polytron Homogenizer (Brinkman Instruments, Westburn,

NJ) and frozen at −20°C until assay. Aliquots of the homogenate were pipetted into oxidizer cups at room temperature for combustion to ^3H_2O and assay by liquid scintillation counting. After overnight drying in the oxidizer cups at room temperature, samples were combusted with a Packard Tricarb Model B306 Oxidizer (Packard Instruments, Downers Grove, IL). Oxidized samples were then assayed for radioactivity in the Packard Tricarb 1500 liquid scintillation counter. Quench correction was performed using external standard counting techniques in both instruments.

Pharmacokinetic Analysis

The terminal disposition rate constant (λ) was estimated by regression of the terminal log-linear concentration time points using PC SAS Version 6.06 (7). AUC_∞ was calculated from the sum of the log-linear trapezoid (linear trapezoidal rule for the ascending portion of the curve and the log trapezoidal rule for the descending portion), $AUC_{0-t'}$ and the extrapolated $AUC_{t-\infty}$ obtained by dividing the last measured L-703,014 plasma concentration by λ. Volume of distribution at steady-state ($V_{d_{ss}}$) was estimated by the method of Perrier and Mayersohn (8). The systematic clearance was also calculated as well as the bioavailability of the orally administered dogs. Actual sampling times were used in all pharmacokinetic calculations. Concentrations labeled as <1.0 ng/mL (below assay sensitivity) were excluded from the pharmacokinetic and pharmacodynamic analysis.

Pharmacodynamics

The ability of L-703,014 to inhibit the *ex vivo* platelet aggregation response to certain agonists (ADP and collagen) is the primary means of describing the activity of this compound. Agonist-induced platelet aggregation studies were performed by a Chronolog aggregometer (Havertown, PA). Collagen-induced platelet aggregation was measured as change in light transmittance in platelet-rich plasma using 10 μg/mL collagen in the presence of 1 μ*M* epinephrine. Collagen-induced platelet aggregation determinations were made at screening, at predose, and 2, 5, 15, 30, 60, 90, 120, and 180 min post bolus i.v. administration of L-703,014 (0.2 mg/kg) and at screening, at predose, and 20, 40, 70, 90, 150, 200, 250, 300, 350, and 480 min after oral administration (2.0 mg/kg). L-703,014 plasma concentrations were recorded at matching time points after both (i.v. and p.o.) administrations. Platelet aggregation (PA_i) measurements at each time "*i*" were converted to inhibition of platelet aggregation (IPA_i) with units of percentage inhibition by the relationship below.

$$IPA_i(\%) = \frac{PA_b - PA_i}{PA_b} \times 100 \quad (1)$$

Baseline platelet aggregation values, PA_b, were determined by calculating the arithmetic mean of the screening and predose platelet aggregation measurements. Values of IPA_i which were calculated as less than 0 were recorded as 0% IPA.

A sigmoid-E_{max} model was chosen to fit the data. The form of this equation is given by Eq. (2), where C_i is the measured L-703,014 plasma concentration, C_{50} is the concentration which yields 50% platelet inhibition, E_{max} is the maximum inhibition obtainable as C_i approaches infinity, and n is a power term that defines the degree of sigmoidicity in the concentration–effect relationship (also referred to as the Hill coefficient).

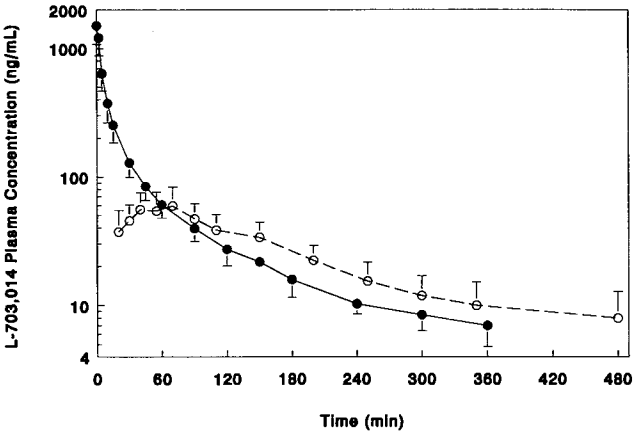


Fig. 2. Mean plasma L-703,014 levels after 0.2 mg/kg bolus i.v. ($C_{p_{i.v.}}$; ●) and 2.0 mg/kg p.o. ($C_{p_{p.o.}}$; ○) administration to dogs (n = 8). Vertical bars indicate one standard deviation.

$$IPA_i = \frac{E_{max} \times C_i^n}{C_{50}^n + C_i^n} \quad (2)$$

The sigmoid-E_{max} model was chosen primarily because the functional relationship explains the sigmoidal nature of the concentration–effect response. Observed L-703,014 plasma concentration–platelet inhibition data were fit to Eq. (2) using the nonlinear least-squares algorithm in PC SAS/STAT

Table I. Pharmacokinetic Parameter Summary After Intravenous (0.2 mg/kg) and Oral (2.0 mg/kg) Administration of L-703,014 to Mongrel Dogs

	0.2 mg/kg i.v. bolus dose			
Dog no.	$AUC_{0-\infty}$ (ng · min/mL)	CL_s (mL/min/kg)	$V_{d_{ss}}$ (L/kg)	$t_{1/2}$ (min)
1	28,476	7	0.49	117
2	27,634	7	0.43	110
3	23,304	9	0.96	204
4	19,313	10	0.63	89
Mean	24,682	8	0.61	118[a]
SD	4,236	2	0.22	36[a]
	2.0 mg/kg p.o. dose			
	$AUC_{0-\infty}$ (ng · min/mL)	C_{max} (ng/mL)	T_{max} (min)	F (%)
1	12,542	66	70	4.4
2	9,345	73	40	3.4
3	11,713	52	40	5.0
4	14,460	102	40	7.5
Mean	11,200	64	50	4.9[b]
SD	1,659	11	17	1.4

[a] Harmonic mean and pseudo standard deviation.
[b] Geometric mean.

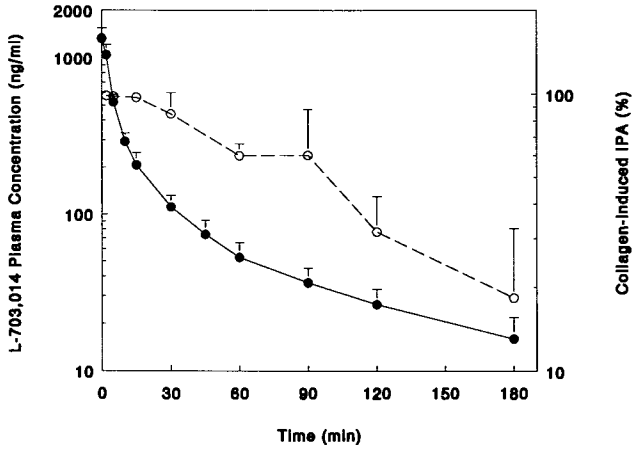


Fig. 3. Double Y-plot of mean L-703,014 plasma concentration (C_p; ●) and mean inhibition of collagen-induced (10 μg/mL) platelet aggregation (IPA; ○) over time following a 0.2 mg/kg intravenous injection to dogs (n = 4). Vertical bars indicate one standard deviation.

with Proc NLIN (7). A Marquardt iterative method was employed to improve convergence. Reciprocal weighting ($1/IPA_i$) was employed to obtain weighted least-squares estimates of E_{max}, C_{50}, and n.

RESULTS

L-703,014 inhibited ADP-induced human gel filtered platelet aggregation with an IC_{50} of 94 nM. It had no significant effect on HUVEC attachment to RGD-containing adhesive proteins until concentrations of 10,000–20,000 μM were achieved. Thus it shows approximately 100-fold selectivity for GP IIb/IIIa over other integrins. Protein binding was constant over the range of 0.01 to 25 μg/mL, with a mean of 23 ± 3.4% bound. Partitioning studies revealed that the mean ratio of plasma to whole blood was 1.22 ± 0.05. Drug concentration and incubation time had no effect on these results as determined by ANOVA.

Figure 2 shows the mean plasma L-703,014 levels after a 0.2 mg/kg bolus intravenous injection and after a 2.0 mg/kg oral administration. The pharmacokinetic parameters from the four dogs in the PK/PD part of these studies are given in Table I. The L-703,014 plasma concentration declined biexponentially. The elimination half-life ranged from 89 to 204 min (mean, 130 min). The mean steady-state volume of distribution was 0.61 L/kg (range, 0.43–0.96 L/kg) and the mean systemic clearance was 8 mL/min/kg (range, 7–10 mL/min/kg). Very little L-703,014 appears systemically after oral administration to the dog. The mean bioavailability from the four dogs in this study was approximately 4.9 ± 1.4%.

Excretion studies with ^{3}H-L-703,014 have shown that L-703,014 is not metabolized and is excreted both in the feces (81% p.o., 70% i.v.) and in the urine (2.2% p.o., 20.3% i.v.) as unchanged drug. Chromatographic characterization of urinary and fecal extracts has verified that unchanged drug was the only significant radioactive component present. An average of 96.0 ± 2.0% of the radioactivity present in the feces (after i.v. or p.o. administration) was ^{3}H-L-703,014. The majority (>90%) of the radioactivity present in the urine after oral administration was ^{3}H-L-703,014. There was a small, more polar metabolite (retention time, ~6 min) present in the urine after oral administration to the dog. No

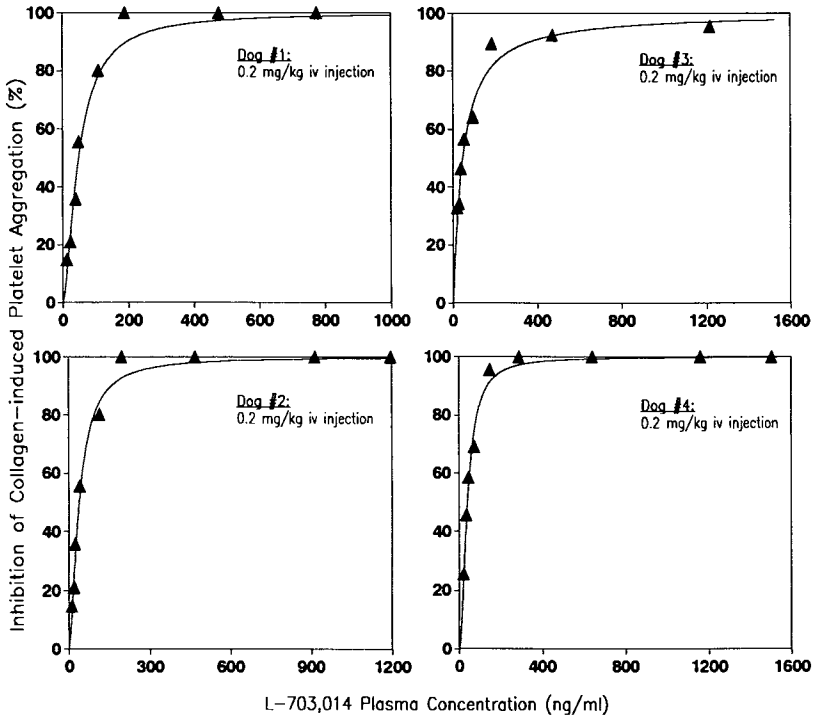


Fig. 4. Inhibition of collagen-induced (10 μg/mL) platelet aggregation versus L-703,014 plasma concentration with fitted sigmoid-E_{max} curve after intravenous administration (0.2 mg/kg) to the dog.

Table II. Pharmacodynamic Parameter Summary After Intravenous (0.2 mg/kg) Administration of L-703,014 to Mongrel Dogs

Dog no.	n	C_{50} (ng/mL)	E_{max} (%)
1	1.54	52.0	105.4
2	1.54	39.6	101.9
3	1.11	46.4	100.0
4	1.75	39.7	101.2
Mean	1.49	44.4	102.1
SD	0.27	6.0	2.3

further characterization of this metabolite has been attempted as yet.

Figure 3 shows a double-Y plot of the mean L-703,014 plasma concentration with the mean IPA profiles versus time from the four dogs in the PK/PD study. The time course of L-703,014 disposition and effect, as measured by inhibition of platelet aggregation using 2 μg/mL collagen as an agonist, are similar. No evidence of hysteresis is present as one would expect from a drug whose effect site is the plasma.

Individual plasma concentration–platelet inhibition profiles with the fitted sigmoid-E_{max} curves for each of the intravenously administered dogs are shown in Fig. 4. Parameter estimates of n, E_{max}, and C_{50} from the fitted data are given in Table II. Figure 4 illustrates the sigmoidal nature of the platelet inhibition response to L-703,014. The mean C_{50} was 44.4 ng/mL (range, 39.6–52 ng/mL), and the mean Hill coefficient was 1.49 (range, 1.11–1.75). Saturability of the effect (100% IPA) was observed in three of the four dogs in the PK/PD study. The mean E_{max} was 102.1 ± 2.3%, indicating that 100% IPA is indeed the observed maximal effect in addition to the functional maximum as dictated by the transformation [Eq. (1)]. Dynamic analysis suggests that L-703,014 is a potent fibrinogen receptor antagonist. The C_{50} value determined in this analysis (98 n*M*) was in strong agreement with the IC_{50} determined *in vitro* for human platelets (94 n*M*).

Figure 5 contains the observed platelet inhibition response in individual dogs after oral administration of L-703,014, along with the i.v. response data and fitted sigmoid-E_{max} curve from the entire i.v. dynamic profile. In light of the small fraction of the oral dose which actually appears systemically, the response data post oral administration are reasonable in comparison with the i.v. observations.

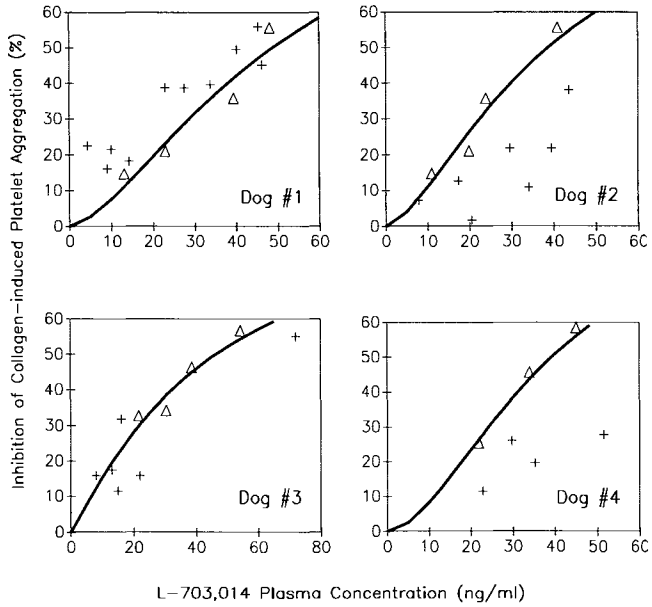


Fig. 5. Inhibition of collagen-induced (10 μg/mL) platelet aggregation versus L-703,014 plasma concentration after oral administration (2.0 mg/kg) to the dog. Sigmoid-E_{max} curve from fitted intravenous dynamic profile (Fig. 4) with i.v. data included for reference (+, p.o.; △, i.v.).

DISCUSSION

The binding of L-703,014 to the fibrinogen receptor is extremely rapid and reversible. Unlike ticlopidine (9–12), the duration of the inhibitory activity of L-703,014 is a function of the drug present in the plasma and not the platelet renewal rate. Hence, global quantitation of the receptor binding using the law of mass action (sigmoid-E_{max} model) is appropriate to describe the pharmacodynamics of this compound.

As L-703,014 is not metabolized, does not exhibit hysteresis, and maintains constant protein binding over the concentration range of pharmacologic interest, the absorption of this compound can be predicted by the levels of collagen-induced inhibition of platelet aggregation. While mean collagen-induced inhibition of platelet aggregation was <40% after administration of 2.0 mg/kg p.o. to the dog, the relationship of L-703,014 plasma concentration to collagen-induced inhibition of platelet aggregation effect was similar to that of the intravenously administered (0.2 mg/kg) dogs. Hence, dynamic modeling of *ex vivo* platelet aggregation would seem to be a viable tool in assessing the oral activity of agents specifically directed against the fibrinogen receptor provided that metabolism, hysteresis, and protein binding are not at issue.

ACKNOWLEDGMENTS

This paper is dedicated to Dr. A. D. Theoharides, a coauthor of this work, who passed away during the completion of the first draft. Tony was a genuine human being who never let politics or personalities hinder his compassion for others or his dedication to science. Although we had known him only a short while, he left a great impression on all of us. We'll miss his participation in late evening chats and we will always remember the way he hiked up his pants before he went to a meeting. Peace in your new life, friend.

REFERENCES

1. E. R. Bates, M. J. McGillem, J. K. Mickelson, B. Pitt, and G. B. J. Mancini. A monoclonal antibody against platelet glycoprotein IIb/IIIa receptor complex prevents platelet aggregation and thrombosis in a canine model of coronary angioplasty. *Circulation* 84:2463–2469 (1991).
2. P. S. Wissel, J. S. Barrett, T. D. Bjornsson, A. Barchowsky, R. J. Gould, E. Hand, D. Farrell, and D. Panebianco. Pharmacokinetics and pharmacodynamic effects of MK-852, a platelet fibrinogen receptor antagonist. *Pharm. Res.* 9:S293 (1992).
3. A. K. Pedersen and G. A. Fitzgerald. The human pharmacology

of platelet inhibition: Pharmacokinetics relevant to drug action. *Circulation* 72:1164–1176 (1985).
4. J. Mordenti and A. Rescigno. Theoretical considerations for the design and development of a drug: Influence of pharmacokinetics and dosage regimen on receptor interactions. *Pharm. Res.* 10:777–782 (1993).
5. G. D. Hartman, M. S. Egbertson, W. Halezenko, W. L. Laswell, M. E. Duggan, R. L. Smith, A. M. Naylor, P. D. Manno, R. J. Lynch, G. Zhang, C. Chang, and R. J. Gould. Non-peptide fibrinogen receptor antagonists. 1. Discovery and design of exosite inhibitors. *J. Med. Chem.* 35:4640–4642 (1992).
6. Z.-R. Gan, R. J. Gould, J. W. Jacobs, P. A. Friedman, and M. A. Polokoff. Echistatin. A potent platelet aggregation inhibitor from the venom of the viper, Echis carinatus. *J. Biol. Chem.* 263:19827–19832 (1988).
7. SAS Institute, Inc. *SAS/STAT User's Guide, Vol. 2, Version 6,* 4th Ed. SAS Institute, Cary, NC, 1990.
8. D. Perrier and M. Mayersohn. Noncompartmental determination of the steady-state volume of distribution for any mode of administration. *J. Pharm. Sci.* 71:372–373 (1982).
9. C. Picard-Fraire. Ticlopidine hydrochloride: Relation between dose, kinetics, plasma concentration and effect on platelet function. *Thromb. Res.* (Suppl.) 4:119–128 (1983).
10. C. Picard-Fraire. Pharmacokinetic and metabolic characteristics of ticlopidine in relation to its inhibitory properties on platelet function. *Agents Actions* 15(Suppl):68–75 (1984).
11. J. J. Thebault, C. E. Blatrix, J. F. Blanchard, and E. A. Panak. Effects of ticlopidine, a new platelet aggregation inhibitor in man. *Clin. Pharmacol. Ther.* 18:485–490 (1975).
12. T. DiPerri, F. L. Pasini, C. Frigerio, P. Blardi, F. Centini, G. L. Messa, A. Ghezzi, and L. Volpi. Pharmacodynamics of ticlopidine in man in relation to plasma and cell concentration. *Eur. J. Clin. Pharmacol.* 41:429–434 (1991).