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Pharmacokinetic and pharmacodynamic analysis of TS-943, a selective non-peptide platelet glycoprotein-IIb/IIIa (GPIIb/IIIa) receptor antagonist, using a nonlinear mixed effect model in dogs

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Abstract

A simultaneous analysis of the pharmacokinetics and pharmacodynamics of TS-943, a selective non-peptide platelet glycoprotein-IIb/IIIa (GPIIb/IIIa) receptor antagonist, was made in dogs using a nonlinear mixed effect model. Plasma concentrations of TS-943 were determined after bolus intravenous injection, constant infusion and bolus plus constant infusion. Pharmacokinetic/pharmacodynamic data were fitted using NONMEM software. The pharmacokinetics of TS-943 fitted a two-compartment open model with first-order elimination. The pharmacodynamic model that best fitted platelet aggregation was an inhibitory sigmoid Emax model. The final estimates for E_0 (baseline effect), Emax (maximum effect), IC50 (50% inhibitory concentration) and γ (Hill coefficient) were 66.3%, 64.3%, 104 ng mL^{-1} and 1.37, respectively. Correlations between TS-943 plasma concentration and extension of template bleeding time were examined by fitting with an exponential model. The TS-943 plasma concentration necessary to double bleeding time (C2-BTE) was approximately 209 ng mL^{-1}. The model estimated that the C2-BTE/IC50 (inhibition of platelet aggregation) ratio was approximately 2.0-fold in dogs. Our results suggest that the ratio values for dogs and man are comparable. A nonlinear mixed effect model was a useful tool for exploring the concentration–effect relationship for both efficacy and safety of TS-943 in dogs and man. In this study, the dog was found to be a useful model for screening of efficacy and safety of TS-943 in man.

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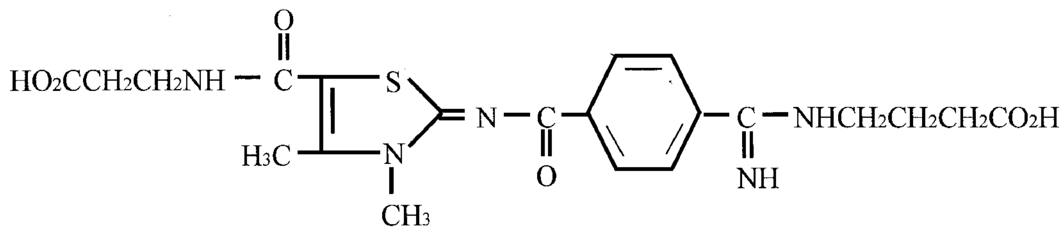
Introduction

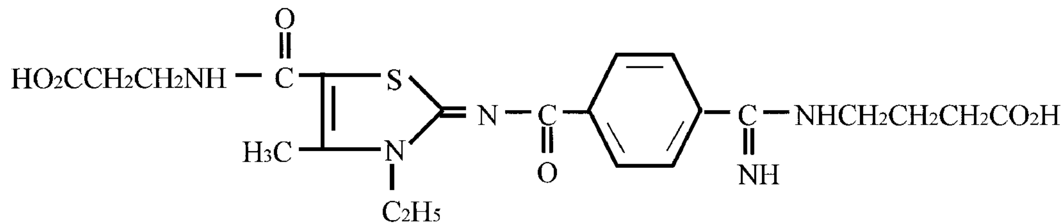
An animal model allows evaluation of drug targets as well as target–disease interactions, and helps to design follow-up experiments as second-generation compounds are studied. It is important to predict concentration–response relationships for efficacy and safety which can be extrapolated from animals to man. Although pharmacokinetic/pharmacodynamic relationships have been established for a number of drugs in both experimental animals and man, there is little information concerning glycoprotein-IIb/IIIa (GPIIb/IIIa) antagonists. In particular, the relationship between pharmacokinetics and pharmacodynamics in laboratory animals remained to be established for the GPIIb/IIIa receptors. A drug that could effectively control unwanted aggregation without adversely affecting bleeding would represent a significant advance in antiplatelet therapy, especially in situations where bleeding is problematic or entirely undesirable.

The anti-thrombotic efficacy of TS-943 (Figure 1), a low-molecular-weight novel antiplatelet agent with high affinity and specificity for GPIIb/IIIa receptors in man, has been described (Furuya et al 2000). There is evidence that the population approaches could be estimated for exploring the concentration–effect relationship for both efficacy and safety.

There is an inherent interindividual variability which may limit the usefulness of the results obtained using traditional pharmacokinetic/pharmacodynamic (PK/PD) modelling approaches, such as the use of mean data for the purpose of prediction. The



TS-943



Internal standard

Figure 1 Chemical structure of TS-943 and the internal standard.

population approach, a data analysis tool that allows for characterization of typical PK/PD profiles related to variability factors, has proven to be useful for such an analysis (Shiner 1984; Flores-Murrita et al 1998).

In this report, we characterized the PK/PD relationship between the effect of platelet inhibition and the adverse event of bleeding time extension in dogs using a nonlinear mixed effect model. At the same time, a comparison was conducted between the pharmacodynamic parameters in dogs and man, with view to using the pharmacodynamic parameters in dogs to predict both efficacy and safety in man.

Materials and Methods

Reagents

Adenosine diphosphate (ADP), adrenaline (epinephrine) and reagents used in these studies were obtained from Sigma Chemical Co. (St Louis, MO). TS-943 (4-{[4-({5-[(2-carboxyethyl)carbamoyl]-3,4-dimethyl-2(3*H*)-thiazolidene}carbamoyl)benzimidoyl]amino}butyric acid) and internal standard (4-{[4-({5-[(2-carboxyethyl)carbamoyl]-3-ethyl,4-methyl-2(3*H*)-thiazolidene}carbamoyl)benzimidoyl]amino}butyric acid) were synthesized at the Taisho Pharmaceutical Co. All other chemicals and solvents were of analytical grade.

Animals

Male hound dogs, 15.5–19.5 kg (Kashou, Tokyou, Japan), used for the experiments were housed in stainless-steel cages and fed once daily with standard laboratory chow. This project was approved by the ethical committee at Taisho Pharmaceutical and was performed in accordance with Recommendations (1987) for the Care and Use of Laboratory Animals.

Study design

Dogs, anaesthetized by administration of 13 mg kg^{-1} Ketalar intramuscularly and 25 mg kg^{-1} Nembutal intraperitoneally, were placed on a respiratory pump (NS-480-3, Sinano Seisakusho, Tokyo, Japan) in which the stroke volume of the respirator pump was adjusted to the dog's weight. An intravenous bolus of TS-943 was then rapidly administered as soon as possible, followed by an infusion of the drug. The antebrachii vein was cannulated to administer TS-943. Bleeding time was determined before and at different intervals after TS-943 administration. Blood samples taken from the jugular vein were collected into 1/10 of 3.8% sodium citrate as anticoagulant before and at different intervals after TS-943 administration. Intravenous injection of the drug was given in a dose of 10, 30, 100 and 300 $\mu g\ kg^{-1}$. Each constant infusion dose of TS-943 was 0.3 $\mu g\ kg^{-1}\ min^{-1}$ for 4 h, 1.0 $\mu g\ kg^{-1}\ min^{-1}$ for 4 h and 3.0 $\mu g\ kg^{-1}\ min^{-1}$ for 4 h, respectively. The bolus plus infusion doses of TS-943 were 30 $\mu g\ kg^{-1}$ plus 1.0 $\mu g\ kg^{-1}\ min^{-1}$ for 4 h, 100 $\mu g\ kg^{-1}$ plus 0.3 $\mu g\ kg^{-1}\ min^{-1}$ for 4 h and 100 $\mu g\ kg^{-1}$ plus 1.0 $\mu g\ kg^{-1}\ min^{-1}$ for 4 h.

Sample preparation

The blood was centrifuged for 10 min at 600 rev min^{-1} at room temperature (SP7D2, HITACH, Tokyo, Japan) to

remove platelet-rich plasma. The remaining blood was centrifuged for 10 min at 3000 rev min^{-1} at room temperature to remove platelet-poor plasma. Plasma concentration was evaluated using the platelet-poor plasma. Platelet-poor plasma samples were extracted by solid-phase extraction on 100-mg CH extraction columns (BOND ELUT; International Sorbent Technology, Mid-Glamorgan, UK) after addition of an internal standard (Figure 1). The column was washed with 3 mL of 0.1 M ammonium acetate solution (pH 4.0) to remove polar species that might otherwise interfere with the analysis. The washings were discarded, and TS-943 and its internal standard were eluted with 3 mL of methanol. The methanolic eluate was evaporated close to dryness and the residue was diluted with 0.2 mL of mobile phase. After passing through a membrane filter (0.22 μm), a 100-μL sample was injected onto the HPLC column.

Measurement of TS-943 plasma concentrations

The concentration of TS-943 was assayed using a validated HPLC system (pump, LC-10AD; auto injector, SCL-10A; UV detector, SPD-10A; Shimadzu Seisakusho, Kyoto, Japan). The column used was a SUPERIOREX ODS S-5 μm (4.6×150 mm, Shiseido, Japan). A mixture of acetonitrile–0.1 M ammonium acetate (13:87) was used as a mobile phase, the elution was at 1.0 mL min^{-1} and 40°C. The peak was detected at UV 346 nm and the area was calculated using an integrator (C-R7A; Shimadzu Seisakusho, Kyoto, Japan). The sensitivity limits of this method were 5–1000 ng mL^{-1} with plasma (plasma containing a higher concentration was diluted before analysis). Recovery of TS-943 and internal standard from plasma was approximately 100%, and within-day and day-to-day coefficients of variation for quality control sample were < 2%.

Platelet aggregation assay

Platelet aggregation was measured using a PAM-8T (Eruma Corp., Tokyo, Japan) by the light transmittance method (Born 1962). In brief, the blood was centrifuged for 7 min at 600 rev min^{-1} at room temperature, aggregation was followed for 5 min after the addition of 10 μM ADP and 10 μM adrenaline into platelet-rich plasma, and the maximum percent increase of light transmission obtained during this period was calculated.

Bleeding time

The effect of TS-943 on bleeding time (BT, min) was assessed on the back of the tongue. A Simplate device (Organon Teknika Corp., Durham, NC) was applied to the surface of the dog tongue to make a uniform incision, and blood was blotted with filter paper at 30-s intervals until bleeding completely stopped; the cut-off time was 30 min. Bleeding time was assessed before and at different intervals after administration of TS-943. The targeted level of bleeding time prolongation was set at 30 min. Extension in bleeding time (BTE) was defined for time i as follows (Barrett et al 1994):

$$BTEi = BTi/BTb \quad (1)$$

in which the baseline measurement (BTb) was taken before the start of administration.

Population pharmacokinetic/pharmacodynamic analysis

A two-compartment model with first-order elimination was used to fit TS-943 plasma concentration–time profiles for all regimens. The interindividual variability in parameters (rate constant of elimination (k_{el}), rate constant from central to peripheral compartment (k_{12}), rate constant from peripheral to central compartment (k_{21}), central volume (V_c)) was modelled as follows:

$$Pj = P \times \exp(\eta j) \quad (2)$$

in which P is the population mean, Pj is the individual parameters for subject j and ηj is the individual random perturbation from the population mean parameter that is independent and is identically distributed with a mean of zero and variance ω^2.

The magnitude of residual variability in plasma concentrations was given as follows:

$$Cpij = Cp_{M}ij \times \exp(\epsilon 1ij) + \epsilon 2ij \quad (3)$$

in which $Cp_{M}ij$ and Cpij are the predicted and observed plasma concentration at time i for an individual j, respectively; $\epsilon 1ij$ and $\epsilon 2ij$ were assumed to be independent random gaussian variables with a mean zero and variable $\sigma^2_{1\epsilon}$ and $\sigma^2_{2\epsilon}$, respectively.

The effect was related to the drug concentration in the central compartment. A site of drug effect was the plasma and there was no reason to suspect hysteresis in deserved data.

The relationship between the observed plasma concentration of TS-943 and observed platelet aggregation response (%) was evaluated using three different pharmacodynamic models (linear, inhibitory maximum effect (Emax) and inhibitory sigmoid Emax model) (Gabrielsson & Weiner 1994) as follows:

$$\text{Effect (inhibitory sigmoid Emax model)} = E_0 - (Emax \times Cp)/(IC50\gamma + Cp\gamma) \quad (4)$$

$$\text{Effect (inhibitory Emax model)} = E_0 - (Emax \times Cp)/(IC50 + Cp) \quad (5)$$

$$\text{Effect (linear model)} = (A1 \times Cp) + A2 \quad (6)$$

in which E_0 is the baseline effect, Emax is the maximum effect observed as Cpi approaches infinity, IC50 is the 50% inhibitory concentration, γ is the Hill coefficient, A1 is the slope and A2 is the intercept.

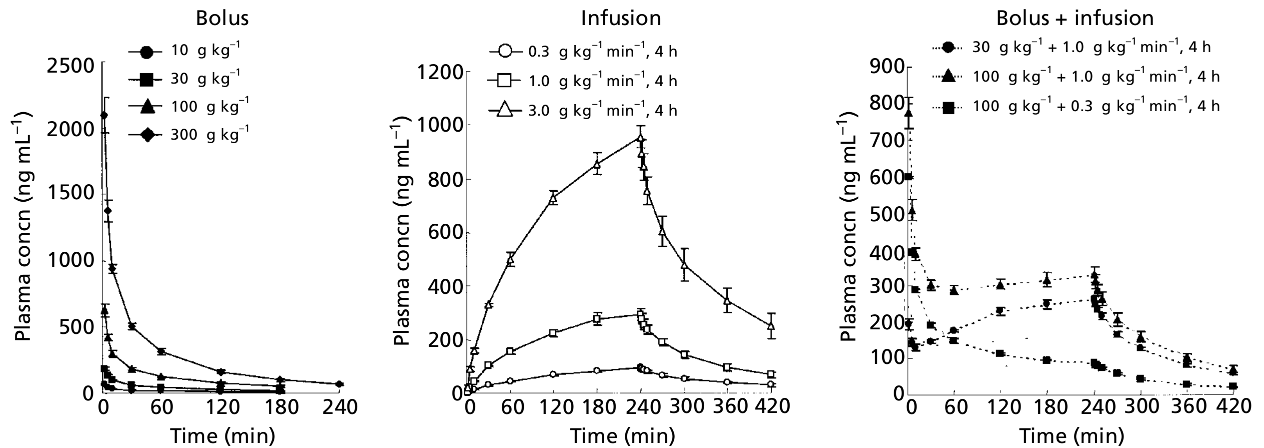


Figure 2 Plasma concentration after administration of TS-943 to dogs.

The interindividual variabilities in parameters of E_0, Emax and IC50 (not for γ) were modelled as follows:

$$Pj = P \times \exp(\eta j) \quad (7)$$

The pharmacodynamic parameters were assumed to be of log normal distribution in the same manner as for pharmacokinetic parameters.

The magnitude of residual variability in the effect on platelet aggregation response (%) was modelled based on the assumption of absolute error as follows:

$$Effectij = Effect_M ij + \epsilon ij \quad (8)$$

The relationship between TS-943 plasma concentration and bleeding time extension was modelled on exponential model studies in man (Barrett et al 1994; Furuya et al 2000). The exponential model was used to fit the BTE and TS-943 plasma concentration data in dogs.

$$BTEi = P1 \times \exp(P2 \times Cp) \quad (9)$$

in which P1 is equivalent to the ordinate intercept and P2 is a shape parameter indicating the steepness of the BTEi-Cpi. A large value of P2 indicates that only a small amount of drug is necessary to cause large increases in BTE, whereas small P2 values reflect the fact that high plasma concentrations are necessary to elevate BTE. Equation 9 can be linearized by taking the natural log of both sides of the equation. An expression for the plasma concentration necessary to double BTE can be derived as follows:

$$C2\text{-}BTE = \ln(2)/P2 \quad (10)$$

C2-BTE is a useful parameter because it gives an indication of the potency of the compound with respect to extension of bleeding time and may help define the therapeutic window for similar agents, provided that the exponential model is appropriate (Barrett et al 1994).

The magnitude of interindividual variabilities, such as pharmacodynamic parameters, were modelled with the assumption of log normal distribution as follows:

$$Pj = P \times \exp(\eta j) \quad (11)$$

The magnitude of residual variability, such as the BTE's, was modelled based on the assumption of log normal distribution as follows:

$$BTEij = BTE_M ij \times \exp(\epsilon ij) \quad (12)$$

All data were analysed using a population model program NONMEM version 5, level 1.0 (Beal & Sheiner 1992) with double precision, installed on a PC-9821Xa 13 personal computer (NEC).

Results

Pharmacokinetics

The pharmacokinetic model selection was based on a preliminary analysis which showed plasma profiles of TS-943 after constant infusion to be best characterized by a two-compartment model and first-order elimination (Figure 2). The population parameters of k_{el}, k_{12}, k_{21} and V_c were 1.49 L h^{-1}, 4.59 L h^{-1}, 2.58 L h^{-1}, and 2.34 L respectively. The interindividual variabilities in k_{el}, k_{21}, k_{21} and V_c were calculated as 21.7, 12.7, 31.3 and 12.0%, respectively, and the residual variability for TS-943 concentration was 7.51% and 4.91 ng mL^{-1}. There was a good agreement between the observed plasma concentrations and the predicted plasma concentrations calculated using NONMEM.

Inhibition of platelet function

The population pharmacodynamic parameters of platelet aggregation are summarized in Table 1. The best fit was obtained with a sigmoid Emax model (Figure 3). The population parameters of E_0, Emax, IC50 and γ were 66.3%, 64.3%, 104 ng mL^{-1} and 1.37, respectively. The interindividual variabilities in Emax and IC50 were calculated to be 12.4 and 71.7%, respectively, and the residual variability for platelet aggregation response was 29.9%. The interindividual variabilities in E_0 were negligible

Table 1 Population pharmacokinetics of TS-943 for platelet aggregation in dogs.

Model	OBJ	E_0 (%)	Emax (%)	IC50 (ng mL^{-1})	γ	ωE_0 (%)	ω_{Emax} (%)	ω_{IC50} (%)	$\omega\gamma$	σ (%)
Sigmoid Emax	2411.270	66.3	64.3	104	1.37	–	12.4	71.7	–	29.9
Emax	2473.303	65.7	86.4	248		21.0	30.6	99.7		27.6
		a	b			ω_a (%)	ω_b (%)			σ (%)
Linear	3185.929	−0.181	34.5			45.4	50.1			79.2

OBJ, the minimum value of objective function (−2log likelihood) in each NONEM run; E_0, baseline value existing in the absence of drug; Emax, maximal drug effect; IC50, plasma concentration yielding 50% inhibition of aggregation; γ, Hill coefficient; a, slope; b, intercept.

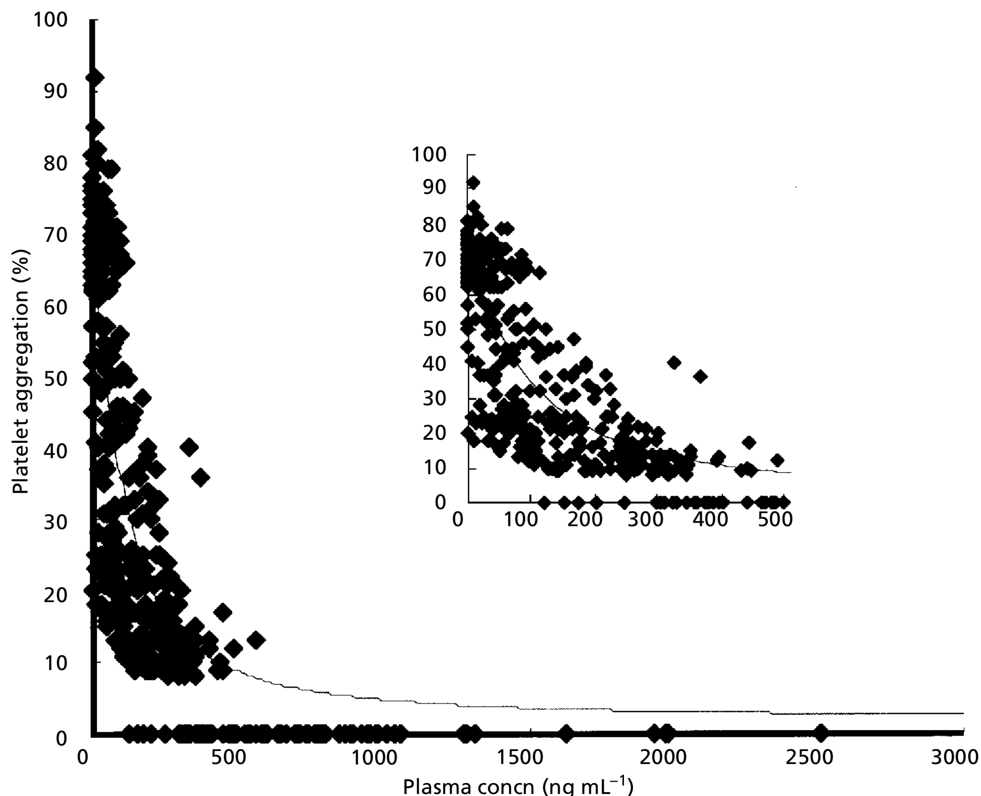


Figure 3 Platelet aggregation (ADP and adrenaline induced (%)) vs plasma concentration after administration of TS-943 to dogs.

(approximately 0.001%), and were removed from the pharmacodynamic model. This had little effect on estimates of parameters or on interindividual and residual variabilities.

The bleeding time

The individual TS-943 concentrations and bleeding time extensions from dogs were fitted to the exponential model (Figure 4). The population parameters of ordinate intercept (P1) and shape parameter (P2) were 1.38 and 0.00332, respectively. The interindividual variabilities in P1 and P2 were calculated to be 50.9 and 37.3%, respectively, and the residual variability for BTE was 1.62. This model estimates that the TS-943 plasma concentration necessary to double BTE is approximately 209 ng mL^{-1}, and the concentration necessary to cause a two-fold BTE is 2.0-fold greater than the IC50 for ADP-induced inhibition of platelet aggregation.

Discussion

The pharmacokinetics of TS-943 in dogs, as well as man, was explained by a two-compartment model. The clearance (CL) and elimination half-life ($t_{1/2}$) values calculated by

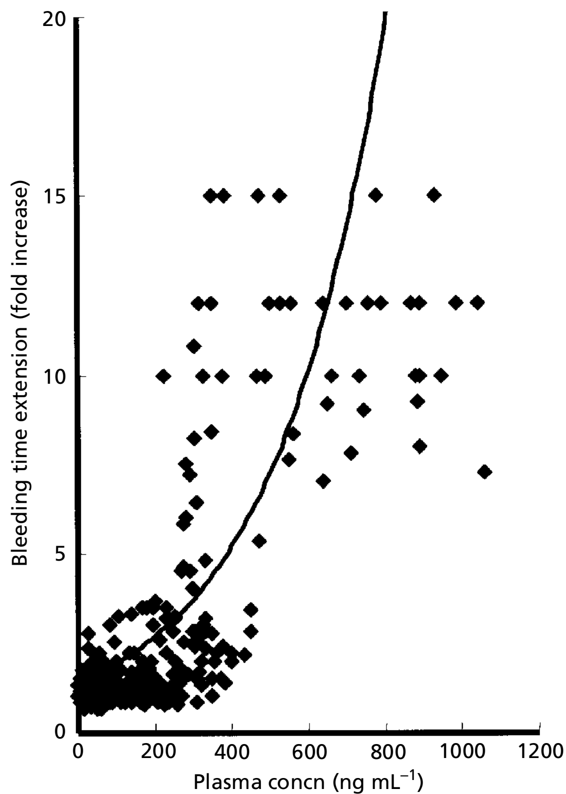


Figure 4 Bleeding time extension vs TS-943 plasma concentration with fitting exponential dynamic model.

NONMEM analysis were 3.49 L h^{-1} and 1.48 h, respectively. A close linear relationship enabled prediction of the observed plasma concentrations from the concentration calculated using NONMEM. There was a good agreement between the pharmacokinetic parameter estimates from the population analysis and those obtained from non-compartmental analysis (CL: 3.35±0.12 L h^{-1}, $t_{1/2}$: 1.74±0.07 h (mean±s.e.)). From population analysis, the value obtained for interindividual variability of pharmacokinetic parameters and residual variabilities was low (within approximately 30%), probably because TS-943 was metabolically stable and its protein binding was found to be low in all species. Thus, in dogs, approximately 90% of the total dose was excreted into the urine as the unchanged parent drug (data not shown). The CL for TS-943 was almost the same as that in man (Furuya et al 2000), although the V_c in man was different from that in dogs.

As a next step, the relationship between the plasma concentration of TS-943 and platelet aggregation response or bleeding time was estimated. Examination of the concentration–effect relationship with drugs that affect platelet function is well known (DiPerri 1991; Barrett et al 1994; Mousa 1996; Michaelis 1998). The antiplatelet effect of TS-943 was shown to follow a concentration-dependent model (Figure 2). TS-943 has several attractive properties that permit pharmacodynamic assessment. An identical counterclockwise hystersis loop was not observed in dogs and man (Furuya et al 2000). The effect compartment was assumed to be equal to the central compartment, and the observed concentration in plasma was directly linked to the effect site concentration. The inhibition of platelet aggregation of TS-943 appeared to be almost dependent on the concentration of unchanged parent drug in plasma because there was little amount of metabolites in plasma. Three different pharmacodynamic models (linear, inhibitory maximum effect (Emax) and inhibitory sigmoid Emax model) were tested to assess the TS-943 plasma concentration at platelet aggregation. The best fit was obtained with an inhibitory sigmoid Emax model in dogs, as in man (Furuya et al 2000). On the other hand, the Emax value of the Emax model exceeded the E_0 value. Goodness-of-fit was not obtained with the Emax model. The population estimates for IC50 was 104 ng mL^{-1} in dogs, whereas in man the IC50 for TS-943 (Furuya et al 2000) was 23.4 ng mL^{-1} ex-vivo, and was approximately 4 times lower than that in dog. The difference in sensitivity between animals and man has been reported for another GPIIb/IIIa receptor antagonist (Refino et al 1998). The IC50 value for inhibition of platelet aggregation of another GPIIb/IIIa receptor antagonist, DMP728, in man (Mousa et al 1996) seemed to be different from that in dogs.

Bleeding time is often measured during clinical examination of an anti-thrombotic agent, and is most often recorded as an independent observation to assess in-vivo platelet function for purposes of safety. Thus, we evaluated the plasma concentration of TS-943 required to cause 80% inhibition of ex-vivo platelet aggregation and also the concentration which caused an increase in bleeding time. TS-943 increased bleeding time in a dose-and concentration-dependent manner in dogs, as well as in man. A small increase was observed in bleeding time at doses that inhibited platelet aggregation response by 80%.

There is the possibility that bleeding times of TS-943 may be prolonged to over 30 min at concentrations exceeding about 300 ng mL^{-1} in dogs. Reversal of the effects caused by TS-943 was essentially complete within a few hours in dogs. Accordingly, the relationship between the observed TS-943 plasma concentration and BTE was determined. The results provided useful information on the tendency for TS-943 to increase bleeding time.

The C2-BTE calculated using equation 10 was approximately 209 ng mL^{-1} in dogs, suggesting that the concentration necessary to cause two-fold BTE is approximately 2.0-fold greater than the IC50 for inhibition of platelet aggregation. The mean C2-BTE/IC50 ratio for TS-943 in dogs was close to that in man (Furuya et al 2000). In general, GPIIb/IIIa receptor blockade is associated with calcium cations. Our inhibition studies have been achieved using dog and human plasma anticoagulated with ethylenediaminetetraacetic acid (EDTA) and Na citrate, respectively. It was reported that the concentration required to inhibit ADP-induced aggregation by 50% was more than 4-fold higher for plasma anticoagulated with PPACK (non-chelating anticoagulant) than for plasma anticoagulated with citrate. In addition, recent studies by Tcheng et al (2001) also showed that receptor occupancy by Integrilin (eptifibatide) also varied according to choice of anticoagulant, with a greater degree of occupancy re-

corded at all points in blood anticoagulated with citrate. It is currently unknown whether the observed effects of chelating anticoagulants on the apparent potency of Integrilin will be observed with TS-943. The platelet-aggregating activity of TS-943 using citrate-anticoagulated blood also might be overestimated, and hence the C2-BTE/IC50 ratios also might vary. However, the degree of platelet GPIIb/IIIa receptor occupancy by eptifibatide in samples collected in PPACK and citrate anticoagulants paralleled the pharmacodynamic observations, as shown by Tcheng et al (2001). Phillips et al (1997) reported that chelation of Ca^{2+} with either citrate or EDTA (chelating anticoagulant) caused a similar degree of enhancement of Integrilin inhibition of ADP-induced platelet aggregation. It is considered that similar C2-BTE/IC50 ratios in dogs and man can be obtained according to the type of anticoagulant used in studies. Analysis of data from dog studies by a nonlinear mixed effect model might be useful for predicting both efficacy and safety of TS-943 in man.

Conclusion

The population approaches could be estimated to explore the concentration–effect relationship for both efficacy and safety in dogs. Marked inhibition of platelet aggregation was observed with doses at which bleeding time was approximately two to three times prolonged above the baseline. A nonlinear mixed effect model effectively linked drug levels with platelet aggregation and the bleeding time seen with all doses given to the dogs. The results from this study suggested that the C2-BTE/IC50 ratios for dogs and man were comparable. The dog might appear to be a good model to predict the efficacy and safety of GPIIb/IIIa antagonists in man, using a nonlinear mixed effect model.

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