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Pharmacokinetics of Ticlopidine in the Rabbit

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Abstract

There is no information about the pharmacokinetics of ticlopidine in rabbits. Such information is valuable in designing appropriate dosing regimens for experimental studies of the drug with ultimate applications in man. The disposition kinetics of ticlopidine at three dose levels were evaluated in three groups of six rabbits which received 10, 50 or 100 mg kg^{-1} drug once daily via the oral-gastric route. Blood samples were collected at predetermined times after the third dose. Plasma concentrations of the unchanged drug were determined by a validated liquid chromatography-mass spectrometry method with a limit of detection of 5 μg L^{-1}.

There was a disproportionate increase in the mean maximum plasma concentration (C_{max}) and the area under the plasma drug-concentration–time curve (AUC) for the 10 and 50 mg kg^{-1} doses. The apparent terminal half-life ($t_{\frac{1}{2}\beta}$), apparent volume of distribution (Vd_β/F), and total plasma clearance (CL_p/F) of the drug were all dose-dependent. For example, $t_{\frac{1}{2}\beta}$ for the 10, 50 and 100 mg kg^{-1} doses were 1·04 ± 0·10, 4·24 ± 1·92 and 12·80 ± 6·35 h, respectively, whereas the Vd_β/F values for the corresponding doses were 214 ± 31, 475 ± 221 and 998 ± 420 L kg^{-1}, respectively.

These results show that the 100-mg kg^{-1} dose produces plasma ticlopidine concentrations similar to those found in man after administration of 250 mg of the drug. It is suggested that 100 mg kg^{-1} might be the appropriate dose of ticlopidine for use in rabbit experimental studies with ultimate application to man.

Ticlopidine, an acidic thienopyridine derivative, has a wide spectrum of platelet anti-aggregating activity in man and several animal species (Defreyn et al 1989). Platelet aggregation has been established as an important factor in the aetiology of arterial-occlusive thrombosis, and ticlopidine has been found effective in various platelet-dependent cardiovascular disease states (Balsano et al 1990). Studies performed in our laboratory have demonstrated that the utility of ticlopidine might also be extended to a potential application in the treatment of infective endocarditis. Administration of the drug with antimicrobial therapy not only caused a reduction in cardiac vegetative weight, but also improved the rate of sterilization in a rabbit model of *Staphylococcus aureus* endocarditis (Nicolau et al 1996, 1998). Although the exact mechanism for the apparent beneficial effect of ticlopidine in endocarditis has not been fully elucidated, it is speculated that the mechanism encompasses more than just antiplatelet activity (Nicolau et al 1998).

The pharmacokinetic dissimilarity between man and laboratory animals is, among other variables, a major limiting factor in extrapolating experimental model results to man. Thus, in the development of experimental protocols with applications in man it is necessary to establish clearly the pharmacokinetic and pharmacological characteristics of the drug in the animal model.

The pharmacokinetics of ticlopidine in man are well documented—drug absorption after oral administration is rapid, with peak concentrations occurring 1–3 h after dosing. The drug is both extensively metabolized and highly bound to plasma protein (Desager 1994). Ticlopidine undergoes non-linear elimination such that repeated administration of the drug results in significant prolongation of its elimination half-life (Shah et al 1991; Desager 1994). Although the rabbit has been

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widely used in the development of in-vivo models for investigation of ticlopidine antiplatelet activity (Bednar et al 1996; Sugidachi et al 1996), there is almost no information on the disposition kinetics of the drug in this animal. Such information is valuable in the design of dosing regimens that yield plasma levels in rabbits similar to therapeutic concentrations of the drug in man. The utilization of man-adapted dosages in animal experimental models is known to optimize the clinical relevance of the results generated. The purpose of this study was to evaluate the pharmacokinetics of ticlopidine in rabbits after administration of different oral doses of the drug.

Materials and Methods

Drug administration and sample collection

Eighteen female New Zealand White rabbits (West Brattleboro, VT), 2–3 kg, were used in this study. The animals were cared for in accordance with the guidelines of the US Department of Health and Human Services (National Institutes of Health 1985). They were housed separately in stainless-steel cages (22 in × 25 in × 16 in; Hoeltge, Cincinnati, OH); water and food (Prolab high fibre rabbit formula, Agway, Syracuse, NY) were freely available, although food was withheld for 6 h during the drug-dosing period (from 3 h before until 3 h after the dose). The animals were divided into three groups of six animals and each group received a dose of 10, 50 or 100 mg kg^{-1} ticlopidine (Sigma, St Louis, MO). The drug was administered in 5 mL water by the oral-gastric route via an orogastric tube, after induction of light anaesthesia with 0·25 mL ketamine–acepromazine (10:1; Ketaset 100 mg mL^{-1} and PromAce 10 mg mL^{-1}, Aveco Fort Dodge, IA). Each dose of the drug was administered once daily and blood samples were taken after the third dose via an indwelling catheter that was placed in the central ear artery. Details of the catheter insertion and blood sampling procedures have been published elsewhere (Marangos et al 1995). Blood samples (1 mL) were drawn into heparinized tubes before the last dose and 0·5, 1·0, 1·5, 2, 3, 6, 12 and 24 h thereafter. Samples were centrifuged at 1000 *g* for 15 min to obtain plasma; this was stored at −80°C until analysis.

Sample and data analysis

The plasma samples were analysed for unchanged ticlopidine by a high-performance liquid chromatography-mass spectrometry (HPLC-MS) method developed and validated at Anapharm (Ste-Foy, Quebec, Canada). Internal standard (imipravine in 100 μL), ammonium hydroxide (100 μL) and extraction solvent (5 mL) were added to the rabbit plasma (250 μL) and the sample was mixed. After centrifugation the organic layer was transferred to a clean tube and the extract was evaporated to dryness. The residue was reconstituted with HPLC mobile phase (250 μL) and 20 μL was injected into a liquid chromatograph equipped with a Phenomenex column. The mobile phase flow-rate was 1 mL min^{-1}. Sample detection was performed with a tandem mass spectrometer (PE Sciex API 365). Quantitation was by the peak-area ratio method and a weighted linear regression was used to determine the concentration of the drug. The assay limit of detection was 5 μg L^{-1} and a standard curve with the linear range 0·0051 to 0·638 mg L^{-1} was used. The intraday and interday coefficients of variation (CV) at the concentrations 0·015, 0·300 and 0·450 mg L^{-1} were all $< 7\%$ ($n = 8$). The accuracy of the assay, assessed as the percentage ratio of the experimentally determined to the actual drug concentration, was 89, 95 and 106% at concentrations of 0·015, 0·300 and 0·450 mg L^{-1}, respectively.

Pharmacokinetic data were evaluated by compartmental and non-compartmental methods. The pharmacokinetic parameters—apparent terminal half-life ($t_{\frac{1}{2}\beta}$), apparent terminal rate constant (β) and area under the plasma drug concentration–time curve (AUC)—were estimated by use of one- or two-compartment models with first-order input and first-order elimination, employing a non-linear least-squares fitting program (PCNONLIN, Statistical Consultant, Lexington, KY). Compartment model selection was based on visual inspection of the fit and use of the correlation between the observed and the calculated concentration. The total plasma clearance (CLp/F) was calculated from the formula Dose/AUC. The apparent terminal-phase volume of distribution (Vd_β/F) was determined from the ratio of CLp/F to β.

The relationships between the pharmacokinetic parameters and the doses were examined by analysis of variance; the Mann-Whitney test was used to evaluate the significance of differences between pairs of data. $P < 0{\cdot}05$ was considered to be indicative of significance.

Results

Figure 1 shows the mean plasma concentrations of ticlopidine after oral administration of the drug to the rabbits. Drug disposition was characterized by a one-compartment model for the 10 and 50 mg kg^{-1}

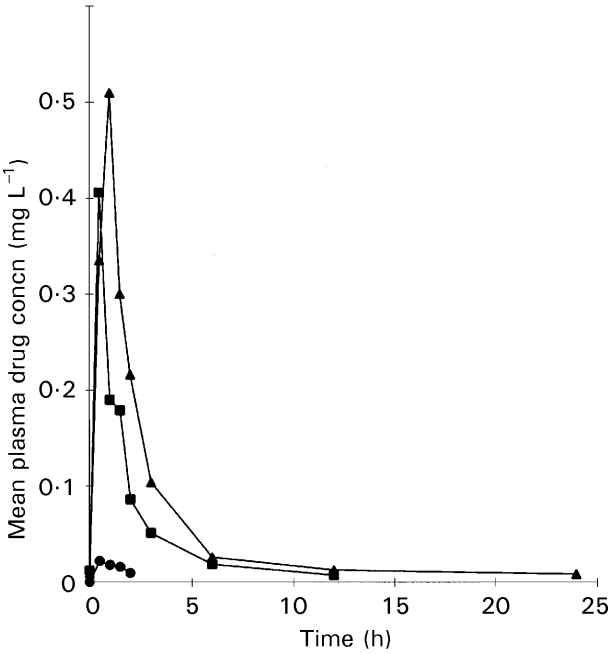

Figure 1. Profiles of mean plasma ticlopidine concentration against time after oral administration of 10 (●), 50 (■) and 100 (▲) mg kg^{-1} doses of the drug separately to three groups of six rabbits.

doses and by a two-compartment model for the 100 mg kg^{-1} dose. All doses of the drug were rapidly absorbed and there was also a very rapid decline in the concentrations such that for the 10 mg kg^{-1} dose plasma levels were below the analytical detection limit 3 h after dosing. The pharmacokinetic parameters derived for the drug are shown in Table 1. The mean kinetic parameters were calculated from individual values first obtained from individual animals. The data in Table 1 are indicative of disproportionate increases in the maximum plasma drug concentration (C_{max}) and in AUC when the dose was increased from 10 to 50 mg kg^{-1}; $t_{\frac{1}{2}\beta}$ increased significantly ($P < 0.05$) with increasing doses. Also, Vd_β/F increased whereas CLp/F decreased with dose; statistically significant ($P < 0.05$) differences between the values of both parameters were observed for the 10 and 100 mg kg^{-1} doses.

Discussion

It is evident from the time of maximum drug concentration, T_{max} (mean value < 1 h for each of the three doses, Figure 1) that ticlopidine absorption occurs rapidly in rabbits. This rapid rate of absorption was dose-independent, which is consistent with the absorption profile of the drug in man (Picard-Fraire 1983; Desager et al 1990; Shah et al 1991). The concentration–time data for all the doses could not be fitted to the same compartment model and this provided the first indication that the drug has dose-dependent kinetics. Evidence of the non-linear kinetics of the drug is clearly provided by the $t_{\frac{1}{2}\beta}$ values, which were significantly ($P < 0.05$) higher for higher doses. This observation marks another similarity between ticlopidine kinetics in man and in rabbits, because the pharmacokinetics of the drug are also non-linear in man, with $t_{\frac{1}{2}\beta}$ increasing with either increasing dose or after multiple dosing (Flores-Runk & Raasch 1993; Buur et al 1997). Although an increase in $t_{\frac{1}{2}\beta}$ might not necessarily result in a decrease in total plasma clearance, our results showed a marked decrease in CLp/F obtained after the 50 or 100 mg kg^{-1} doses in comparison with that for the 10 mg kg^{-1} dose. There was no difference between CLp/F for the 50 and 100 mg kg^{-1} doses. This is attributable to an increase in V_β/F obtained with the 100-mg kg^{-1} dose which is also associated with a correspondingly lower β value (because $CLp = \beta \times Vd_\beta$). Thus, the occurrence of dose-dependent disposition was manifested in the Vd_β/F values (Table 1). The increase in Vd_β/F with dose might result from saturation of the sites of binding

Table 1. Pharmacokinetic parameters of ticlopidine after oral administration of various doses of the drug to rabbits.

Dose	T_{max}	C_{max}	AUC	$t_{\frac{1}{2}\beta}$	Vd_β/F	CLp/F
10	0·80 ± 0·40	0·027 ± 0·008	0·071 ± 0·003	1·04 ± 0·10*	214 ± 31†	141 ± 6†
50	0·88 ± 0·41	0·425 ± 0·376	0·875 ± 0·325	4·24 ± 1·92*	475 ± 221	70 ± 34
100	0·83 ± 0·37	0·485 ± 0·250	1·552 ± 0·844	12·80 ± 6·35	998 ± 420	84 ± 40

Values are means ± standard deviations. Kinetic values were determined from individual rabbits (n = 6 for each dose of drug). T_{max} = time of mean maximum plasma concentration (h); C_{max} = mean maximum plasma concentration (mg L^{-1}); AUC = area under the plasma drug concentration–time curve (mg h L^{-1}); $t_{\frac{1}{2}\beta}$ = apparent terminal half-life (h); Vd_β/F = apparent volume of distribution (L kg^{-1}); CLp/F = total plasma clearance (L h^{-1} kg^{-1}). * $P < 0.05$, compared with 100 mg kg^{-1}; † $P < 0.05$, compared with 50 and 100 mg kg^{-1}.

of the drug to plasma proteins, because ticlopidine is known to be extensively bound to plasma protein. A similar postulate has been used to explain the positive correlation between dose and volume of distribution for aspirin (Levy & Yaffe 1974). Although studies in man of plasma protein binding of ticlopidine suggested no marked saturation for the major binding components (Glasson et al 1982), this situation might not be the same in rabbit plasma. In general, non-linear drug pharmacokinetics, as observed in this study, are indicative of the occurrence of saturable binding sites, saturable metabolic or excretion processes, or inhibition of its own metabolism, or a combination of these. The extent to which all these factors contribute to the non-linear kinetics of ticlopidine is yet to be fully elucidated. Saturable first-pass metabolism of the drug has been demonstrated by use of an in-vitro diffusion cell system which directly measured the permeation of ticlopidine across different segments of rabbit intestine (Grass & Bozarth 1994). Such an occurrence seems to largely explain the disproportionate increases in C_{max} and AUC of ticlopidine with increases in the dose.

In this study the 100-mg kg^{-1} dose resulted in plasma concentrations that simulate the profiles obtained in man after administration of a 250-mg dose, which is the unit treatment dose of the drug. For example, the C_{max} of $0{\cdot}482 \pm 0{\cdot}250$ mg L^{-1} and the AUC of $1{\cdot}552 \pm 0{\cdot}844$ mg h L^{-1} are remarkably within the range of values obtained in man after a 250-mg dose (Shah et al 1991, $C_{max} = 0{\cdot}41 \pm 0{\cdot}24$ mg L^{-1}, AUC $= 1{\cdot}40 \pm 0{\cdot}80$ mg h L^{-1}; Buur et al 1997, $C_{max} = 0{\cdot}37 \pm 0{\cdot}24$ mg L^{-1}, AUC $= 1{\cdot}70 \pm 1{\cdot}10$ mg h L^{-1}).

This finding suggests that in rabbit models of experimental ticlopidine antiplatelet activity, results obtained with the use of a 100-mg kg^{-1} dose might have more relevance to application in man, although further studies are necessary to establish whether this observed similarity in the plasma concentration profiles of the drug in man and rabbits is also obtained during long-term drug treatment. Also, because the pharmacologically active drug is the unbound fraction, a knowledge of the difference in the extent of plasma-protein binding of ticlopidine in rabbits and in man would be useful for optimization of pharmacodynamic simulations.

The pharmacokinetic data presented in this report are valuable for designing appropriate dosage regimens of ticlopidine to be used in rabbit models of experimental studies, with ultimate application to man.

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