Lack of effect of ponsinomycin on the pharmacokinetics of nicoumalone enantiomers

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Pharmacokinetic interaction between ponsinomycin-nicoumalone was studied in six subjects who received an 8 mg oral dose of racemic nicoumalone alone and 4 days into an oral regimen of ponsinomycin 800 mg twice daily. The concentrations of R(+) and S(–)-nicoumalone in plasma were measured using a stereospecific h.p.l.c. assay. The disposition characteristics of nicoumalone enantiomers in the control phase of this study were similar to those reported previously with the exception of the data for one subject whose oral clearance for S(–)-nicoumalone was seven times lower than those in the other subjects. A statistically significant effect of ponsinomycin on the kinetics of R(+) and S(–)-nicoumalone was not demonstrated.

Keywords ponsinomycin nicoumalone pharmacokinetics drug interactions
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Introduction

The macrolide antibiotics have the potential to inhibit the metabolism of co-administered drugs and may, therefore, be associated with clinically significant drug interactions (Ludden, 1985). Ponsinomycin, previously named miocamycin, is a new semi-synthetic macrolide. It is extensively metabolized *in vivo* (Couet *et al.*, 1989a, 1990a; Fucaya *et al.*, 1981; Schomura *et al.*, 1981), and while it has no or only minor effect on theophylline disposition (Couet *et al.*, 1989b; Principi *et al.*, 1987), it has been shown to alter the kinetics of carbamazepine (Couet *et al.*, 1990b), cyclosporin (Couet *et al.*, 1990c) and dihydroergotamine (Couet *et al.*, 1990d). Limited observations suggested that macrolides may interfere with the pharmacokinetics of oral anticoagulants (Ludden, 1985), and administration of macrolides to patients treated with oral anticoagulants is currently considered to be potentially hazardous. The aim of this study was to investigate the potential effect of ponsinomycin at steady-state on the pharmacokinetics of nicoumalone enantiomers.

Methods

Clinical

Six healthy male volunteers aged 22–23 years gave their written informed consent to the study which was approved by the Local Ethics Committee. None was taking any other drug and the consumption of alcohol was stopped 12 h prior to and for the duration of the trial. After an overnight fast each subject took at 08.00 h on Day 1, a single 8 mg oral dose of nicoumalone (2 × 4 mg tablets of Sintrom® batch 381, Laboratoires Ciba-Geigy, Rueil-Malmaison, France) with 200 ml of tap water. Venous blood samples were collected immediately prior to and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 9, 12, 24, 36, 48, 60, 72 and 96 h after nicoumalone administration. At 08.00 h on the morning of Day 10, ponsinomycin treatment (800 mg twice daily; at 08.00 h and 20.00 h, with ponsinomycin syrup, batch P4094, Laboratoires Roger-Bellon, Neuilly, Paris, France) was begun. The last dose of ponsinomycin was given on Day 18 at 20.00 h. On the morning of Day 14, when

ponsinomycin was at steady-state (Couet *et al.*, 1990a), each subject took a single 8 mg oral dose of nicoumalone under identical conditions to those observed on Day 1. Blood sampling was carried out as in the control phase. Subjects were admitted to the clinical centre from 12 h before and up to 96 h after each administration of nicoumalone.

Blood coagulation parameters

Prothrombin time was measured using a commercial reagent (Thromboplastine Mérieux, Lyon, France), from time 0 up to 96 h following each administration of nicoumalone, at 24 h intervals.

Analytical methods

Nicoumalone enantiomers were measured simultaneously in plasma using a stereospecific h.p.l.c. assay (Gill *et al.*, 1988). The only modification to the published assay was that commercially available 4'-chlorowarfarin (Aldrich Chimie, Strasbourg, France) was used as an internal standard, instead of 4'-fluorowarfarin. The second eluting peak of the internal standard (retention time = 10.7 min) was used for calculation of S(-)-nicoumalone (retention time = 12.2 min) and R(+)-nicoumalone (retention time = 19.4 min) concentrations. The limit of measurement of the assay was 25 ng ml⁻¹ for both enantiomers. The interassay precision estimated at concentrations of 25 and 500 ng ml⁻¹ was 6.1% and 3.7% respectively for R(+)-nicoumalone and 4.2% and 0.8% for S(-)-nicoumalone. When S(-) or R(+)-nicoumalone were reacted individually with carbobenzyloxy-L-proline, only a single peak was observed, suggesting an optical purity of at least 98.5%.

Estimation of pharmacokinetic parameters

Peak plasma drug concentrations ($C_{\max}$) and time of peak ($t_{\max}$) were observed values. The absorption lag-time (t_{lag}), was taken as the time to the last sample before the drug became detectable in plasma. The total area under the concentration-time curve for each nicoumalone enantiomer (AUC) was determined by the linear trapezoidal rule with extrapolation to infinity. The terminal elimination half-life ($t_{1/2}$) was calculated from $\ln 2/\lambda_z$, where λ_z is the terminal elimination rate constant, determined by linear regression analysis of the log concentration vs time data. For each enantiomer, the apparent clearance (CL/F) was calculated by division of the dose administered (4 mg) by the AUC of the

Figure 1 Plasma concentration-time profiles of R(+)-nicoumalone (a) and S(-)-nicoumalone (b) following a single 8 mg oral dose of racemic drug in a representative subject during the control (○) and ponsinomycin (●) phases of the study.

corresponding enantiomer. A Wilcoxon test was used to compare mean $t_{\max}$ and t_{lag} values, and Student's *t*-test was used to compare mean values of $C_{\max}$, AUC, CL/F and $t_{1/2}$ calculated in the control phase of the study and in the presence of ponsinomycin. A value of $P < 0.05$ was taken as statistically significant.

Results

The results obtained in five of the six subjects are presented together and those obtained in the remaining volunteer (subject 6) are presented separately.

After oral administration of nicoumalone as a racemate to subjects 1 to 5, the plasma concentration-time curves of R(+)-nicoumalone and S(-)-nicoumalone were similar before and after administration of ponsinomycin. Those of R(+)-nicoumalone were mostly characterized by a lag-time followed by a rapid absorption phase and then by a monoexponential decay (Figure 1a). Plasma concentrations of S(-)-nicoumalone were much lower than those of R(+)-nicoumalone and could be measured only up to 4 h, making estimation of $t_{1/2}$ inaccurate (Figure 1b and Table 1). No statistically signifi-

Table 1 Summary of pharmacokinetic data for R(+) and S(-)-nicoumalone (mean $\pm$ s.e. mean) in five subjects following a single 8 mg oral dose of racemate; values obtained for subject 6 are presented in brackets.

	Optical isomer	Control phase	Ponsinomyacin phase	
$C_{\max}$ (ng ml ⁻¹)	R(+)	310 $\pm$ 83 (351)	318 $\pm$ 35 (311)	NS*
	S(-)	112 $\pm$ 14 (185)	113 $\pm$ 29 (173)	NS*
$t_{\max}$ (h)	R(+)	2.1 $\pm$ 0.7 (2.5)	2.4 $\pm$ 0.4 (2.5)	NS**
	S(-)	1.8 $\pm$ 0.8 (2.0)	2.10 $\pm$ 0.4 (3.0)	NS**
t_{lag} (h)	R(+)	0.35 $\pm$ 0.38 (0)	0.45 $\pm$ 0.33 (0.75)	NS**
	S(-)	0.75 $\pm$ 0.61 (0.25)	0.95 $\pm$ 0.37 (0.75)	NS**
AUC (ng ml ⁻¹ h)	R(+)	2919 $\pm$ 607 (3745)	2776 $\pm$ 98 (3657)	NS*
	S(-)#	193 $\pm$ 38 (1344)	193 $\pm$ 40 (1390)	NS*
CL/F (l h ⁻¹)	R(+)	1.43 $\pm$ 0.40 (1.07)	1.44 $\pm$ 0.05 (1.09)	NS*
	S(-)	21.3 $\pm$ 3.8 (2.98)	21.3 $\pm$ 3.6 (2.88)	NS*
$t_{1/2}$ (h)	R(+)	6.96 $\pm$ 1.68 (7.99)	6.35 $\pm$ 0.75 (7.45)	NS*
	S(-)	NR (4.46)	NR (4.89)	

NR: Not reported

*: Student's *t*-test; **: Wilcoxon test.

#: $t_{1/2}$ was not reported for S(-)-nicoumalone because it could not be determined accurately. However, total AUC values were estimated because the fraction extrapolated to infinity using λ_z value was only 7 to 22%.

cant differences were found between the pharmacokinetic parameters of nicoumalone enantiomers ($n = 5$ subjects) in the control phase and in the presence of ponsinomyacin (Table 1).

Ponsinomyacin appeared to have no effect on the kinetics of R(+) and S(-)-nicoumalone in subject 6. However, whereas the disposition of the R(+) enantiomer in this subject was consistent with that observed in other subjects, that of the S(-) enantiomer was markedly different as reflected by the values of the pharmacokinetic parameters (Table 1).

No side effects were reported in the study. Prothrombin time reached a minimum value of 63% of normal in two subjects (including subject 6), and did not fall below 80% in the others.

Discussion

The study protocol that we used was almost identical to that used by Gill *et al.* (1989) to investigate the pharmacokinetic interaction between cimetidine and nicoumalone, with the exception that we administered 8 mg of nicoumalone as a racemate (corresponding to two tablets), instead of 10 mg (2.5 tablets). Accordingly, results obtained in the control phases of these two studies were similar. The mean AUC of S(-)-nicoumalone was on average 15 times less than that of R(+)-nicoumalone in subjects 1 to 5, which compares with the 10-fold difference reported by others (Gill *et al.*, 1989; Godbillon *et al.*, 1981). In most subjects an absorption lag-time was noted, which was not reported by Gill

et al. (1989), and which seemed to be longer for S(-) than for R(+)-nicoumalone. Another difference between our results and those of Gill *et al.* (1989), was that we observed a mono-exponential rather than a biexponential decay of R(+)-nicoumalone plasma concentrations with time.

The most striking finding however, was much higher plasma concentrations of S(-)-nicoumalone in subject 6. The AUC was much higher and CL/F much lower in this subject than in others (Table 1). Because it can be assumed that bioavailability of S(-)-nicoumalone is essentially complete (Godbillon *et al.*, 1981), the low value of CL/F estimated in subject 6 would be due to a decreased metabolic clearance of this enantiomer (Dieterle *et al.*, 1977). Although it must remain only an hypothesis, stereospecific polymorphic metabolism might explain the observation. Such a phenomenon could be clinically relevant because S(-)-nicoumalone possesses an intrinsic activity comparable with

that of R(+)-nicoumalone (Thijssen *et al.*, 1986), and is usually considered to be devoid of activity *in vivo* only because of its rapid elimination (Godbillon *et al.*, 1981; Thijssen *et al.*, 1986).

The results of the present study suggest that ponsinomycin does not interact kinetically with the enantiomers of nicoumalone. However, the possibility of a pharmacodynamic interaction cannot be excluded. An appropriate pharmacodynamic study is difficult to do in healthy subjects since in order to mimic clinical conditions it would be necessary to stabilize the prothrombin time at 25–35%. This would have required administration of therapeutic doses of nicoumalone for several weeks to healthy volunteers (Hoglund *et al.*, 1989; Paulsen *et al.*, 1988; Thijssen & Hamulyak, 1989), which would have raised ethical problems.

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