Stereoselective pharmacokinetics of pazinaclone, a new non-benzodiazepine anxiolytic, and its active metabolite in healthy subjects

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- 1 Serum and urine concentrations of enantiomers of pazinaclone (DN-2327) and an active metabolite M_{II} , were measured after single and twice daily oral doses of 4 and 8 mg racemic drug to healthy subjects.
- 2 The kinetics of *rac*-pazinaclone and *rac*- M_{II} were dose-independent and no unchanged drug was recovered in urine.
- 3 The terminal elimination half-lives of the drug isomers were similar (about 10.5 h), but mean steady-state values of AUC were twofold higher for the *S*-isomer than those of the antipode (e.g., 8 mg dose: 127 vs 69 ng ml⁻¹ h). However, the corresponding AUC values based upon unbound drug were similar (5.71 vs 5.73 ng ml⁻¹ h) indicating no stereoselectivity in intrinsic metabolic clearance.
- 4 The terminal elimination half-lives of *S*- and *R*- M_{II} were similar to those of parent compound indicating that the elimination of these metabolites is formation rate-limited.
- 5 The *R*:*S*-ratio for the AUCs of M_{II} was 4:1. Both enantiomers were excreted in the urine mainly as glucuronide conjugates, with stereoselectivity toward *S*- M_{II} .
- 6 Since only the *S*-enantiomers of DN-2327 and M_{II} bind to the benzodiazepine receptor, further measurements of drug effect in patients should be related to combine serum concentrations of the *S*-enantiomers of both parent drug and M_{II} .

Keywords anxiolytic pazinaclone stereoselective pharmacokinetics glucuronidation

Introduction

Pazinaclone (DN-2327, [(±) 2-(7-chloro-1,8-naphthyridine-2-yl)-3-[(1,4-dioxo-8-azaspiro [4.5] dec -8-yl) carbonyl-methyl]isoindolin-1-one]) is a new non-benzodiazepine anxiolytic with a binding affinity for benzodiazepine receptors, unaffected by GABA, exceeding that of diazepam by a factor of twenty [1]. The specificity of the pharmacological activity of pazinaclone was confirmed by inhibition with the benzodiazepine receptor antagonist Ro15-1788 [2]. Also, it was suggested that pazinaclone acts as an agonist with respect to the anti-conflict and anti-convulsant effects, but as an antagonist to the muscle-relaxant and sedative effects of diazepam. In animal models the anxiolytic effects of pazinaclone were antagonised by flumazenil [3] and it did not impair

acquisition or working memory [4]. In humans, the effects of pazinaclone on the EEG were more related to those of benzodiazepines than buspirone [5].

The proposed metabolic pathway of pazinaclone is shown in Figure 1. Thus, it hydrolyses to an intermediate metabolite, M_I , which is rapidly reduced to a pharmacologically active metabolite, M_{II} . Approximately 20% of the dose is excreted in urine mainly as M_{II} -glucuronide (6). Pharmacological activity, as measured both *in vitro* and *in vivo*, resides primarily in the *S*-enantiomers of pazinaclone and M_{II} (unpublished data). We now report on the pharmacokinetics of the isomers of pazinaclone and its M_{II} metabolite in healthy subjects after single and multiple doses of *rac*-pazinaclone.

Figure 1 Proposed metabolic pathway for pazinaclone in humans. *indicates the chiral centre.

Methods

Subjects and protocol

Twenty-four healthy male adults (age: 19–37 years; weight: 63.4–97.5 kg) participated in the study. Informed consent was obtained from all participants and the study was approved by the Pharmacology Institutional Review Board (Austin, TX), where the study was carried out. Subjects were divided into two groups of 12. The first group received single and multiple doses of 4 mg *rac*-pazinaclone and the second group received single and multiple doses of 8 mg *rac*-pazinaclone. Mean age and weight did not differ ($P > 0.05$) between groups.

On Day 1, subjects were randomised to receive a single tablet of either 4 or 8 mg of *rac*-pazinaclone. For the next 12 days the dose was given twice a day (every 12 h) and on Day 14 a final single dose was given. The first and final single doses were administered at 08.00 h following an overnight fast, which continued for 4 h until lunch was served. All subjects remained in the clinical facility for the entire study period, during which no xanthine containing beverages or foods, other drugs, alcohol, vigorous exercise, and smoking were allowed. The interval between breakfast and lunch was 5 h and that between lunch and dinner was 7 h.

Venous blood samples (10 ml) were collected over 24-h on Days 1 and 14 and additional samples were collected on Days 3 to 13 immediately before either the morning or evening dose. Blood samples were centrifuged (1000 g) at 4–7° C and serum was stored at –20° C until assay. Urine was collected before dosing and for 24 h after the first and final doses and aliquots were stored at –20° C until assay.

Drug analysis

Serum and urine concentrations of pazinaclone, M_{II}, and their respective *S*- and *R*-enantiomers were measured by h.p.l.c. as described elsewhere [7, 8]. For each analyte, the limit of determination was 0.1 and 1 ng ml^{–1} in serum

and urine, respectively. Coefficients of variation were within 10%.

Partition and protein binding studies

Blood-plasma concentration ratios of [¹⁴C]-*rac*-pazinaclone and [¹⁴C]-*S*-pazinaclone were measured at 37° C after incubating each compound (10–500 ng ml^{–1}) for 1 h in four samples of fresh heparinised blood from four healthy subjects. Duplicate aliquots of whole blood and plasma samples were placed in Cumbusto-Cones® (Packard) containing cellulose powder and burned in a Packard Tri-Carb® Model 307 Sample Oxidiser. The ¹⁴CO₂ released was trapped in Carbo-Sorb® and assayed by liquid scintillation as described below.

The binding at 37° C of [¹⁴C]-*rac*-pazinaclone, [¹⁴C]-*S*-pazinaclone, [³H]-*rac*-M_{II}, and [³H]-*S*-M_{II} was determined at concentrations of 10–500 ng ml^{–1} in plasma from four healthy subjects using a Spectrum Equilibrium Dialysis System® with 1-ml cells and a Spectra/Por 2 membrane with a molecular weight cut-off of 12000–14000. The plasma binding of [¹⁴C]-*S*-pazinaclone (20 ng ml^{–1}) was also determined in the presence of 10 and 20 ng ml^{–1} of *R*-pazinaclone, in the presence of 30 ng ml^{–1} of *rac*-M_{II}, and in the presence of 6 ng ml^{–1} of *S*-M_{II} and 24 ng ml^{–1} of *R*-M_{II}. Duplicate aliquots of plasma and buffer samples were added to Insta-Gel® scintillation cocktail and counted in a Tri-Carb® Model 2500TR Liquid Scintillation Analyzer. Correction for quenching was made by automatic external standardization.

Radiolabeled *R*-DN-2327 and *R*-M_{II} were not available. Therefore, based on mass balance, the blood-plasma concentration ratios and *f*_u values for *R*-pazinaclone and *R*-M_{II} were estimated from the values for the respective racemic compound and *S*-isomer.

Pharmacokinetic analysis

Values of *C*_{max}, *t*_{max}, and *C*_{min} were noted directly from the data. Values of AUC(0,12) and AUC(0,24) were

computed by the linear trapezoidal rule. For single doses, total AUC was calculated as the sum of AUC(0,24) and the quotient of the last measurable concentration divided by λ_z . The terminal phase half-life ($t_{1/2,z}$) was calculated as $(\ln 2/\lambda_z)$. The renal clearance (CL_R) of M_{II} was calculated from the urinary recovery of unconjugated metabolite divided by the corresponding AUC.

Statistical analyses

Within each dose group, a paired *t*-test was used to test for differences between single-dose and steady-state kinetic parameters for *rac*-pazinaclone and M_{II} , between those of the *S*- and *R*-enantiomers of pazinaclone and M_{II} at steady-state, and between morning and evening C_{min} values on the last 2 days of twice daily dosing. Differences between dose groups were tested by one-way ANOVA, in which dose proportionality was examined for C_{max} and AUC after dose normalization and all other dose-group differences were taken as the simple differences. All *P* values ≤ 0.050 were considered to be statistically significant. Also, non-parametric 95% confidence intervals (CI) were calculated to assess the statistical significance of differences in the kinetic

parameters of the *S*- and *R*-enantiomers of pazinaclone and M_{II} within each dose group, as well as of differences in protein binding.

Results

Mean pharmacokinetic parameters are summarised in Table 1 and mean serum concentrations of the isomers of pazinaclone and M_{II} on Day 14 of twice daily dosing are shown in Figure 2. For *rac*-pazinaclone and M_{II} , mean $t_{1/2,z}$ values (about 10.5 h) were independent of dose and were similar after single and multiple doses. The accumulation ratios for pazinaclone and M_{II} were about 1.8, irrespective of dose. Morning C_{min} values for both pazinaclone and M_{II} were about 50% higher than those in the evening (data not shown). Unchanged drug was not detected in the urine, whereas M_{II} was excreted in both free and conjugated forms, accounting for less than 1% and 17%, respectively, of the dose.

Regardless of dose, serum concentrations of *S*-pazinaclone were parallel to and approximately twofold greater than those of the *R*-enantiomer. Serum concentrations of *R*- M_{II} were parallel to and four-fold higher than those of *S*- M_{II} . Within each dose-group mean C_{max}

Table 1 Pharmacokinetic parameters[□] of *rac*-pazinaclone, M_{II} metabolite and their *S*- and *R*-enantiomers

	Single dose		Steady-state		
	<i>rac</i> -pazinaclone	<i>rac</i> -pazinaclone	<i>S</i> -pazinaclone	<i>R</i> -pazinaclone	S/R-ratio
4 mg dose group					
C_{max} (ng ml ⁻¹)	11.2 $\pm$ 3.4	21.0 $\pm$ 6.6*	13.2 $\pm$ 4.3	8.1 $\pm$ 3.0 [†]	1.70 {1.36–2.03}
t_{max} (h)	1.0 (1.0–1.5)	1.0 (0.5–2.0)	1.0 (0.5–2.0)	1.0 (0.5–2.0)	
AUC ^a (ng ml ⁻¹ h)	68 $\pm$ 212	113 $\pm$ 39*	73 $\pm$ 28	39 $\pm$ 18 [†]	1.95 {1.57–2.31}
$t_{1/2,z}$ (h)	ND	9.8 $\pm$ 3.1	10.5 $\pm$ 4.1	9.6 $\pm$ 3.8	1.23 {0.93–1.52}
	<i>rac</i> - M_{II}	<i>rac</i> - M_{II}	<i>S</i> - M_{II}	<i>R</i> - M_{II}	
C_{max} (ng ml ⁻¹)	14.0 $\pm$ 4.8	24.1 $\pm$ 5.8*	5.1 $\pm$ 2.4	19.7 $\pm$ 3.7 [†]	0.25 {0.19–0.31}
t_{max} (h)	1.8 (1.0–3.0)	1.5 (1.0–4.0)	1.5 (1.0–3.0)	2.0 (1.0–4.0)	
AUC [‡] (ng ml ⁻¹ h)	102 $\pm$ 43	153 $\pm$ 45*	31 $\pm$ 16	123 $\pm$ 36 [†]	0.25 {0.19–0.31}
$t_{1/2,z}$ (h)	ND	7.6 $\pm$ 2.3	7.6 $\pm$ 3.3	10.7 $\pm$ 7.7	0.81 {0.65–0.97}
CL_R (ml min ⁻¹)	3.84 $\pm$ 2.94	2.55 $\pm$ 1.89	2.08 $\pm$ 1.22	2.68 $\pm$ 1.37	0.78 {0.61–0.95}
Ae (% Dose)					
Free	0.48 $\pm$ 0.43	0.65 $\pm$ 0.54	0.09 $\pm$ 0.03	0.52 $\pm$ 0.24 [†]	0.19 {0.14–0.23}
Conjugated	9.6 $\pm$ 3.6	13.9 $\pm$ 5.6*	10.3 $\pm$ 4.2	2.8 $\pm$ 1.4 [†]	4.07 {3.01–5.11}
8 mg dose group					
	<i>rac</i> -pazinaclone	<i>rac</i> -pazinaclone	<i>S</i> -pazinaclone	<i>R</i> -pazinaclone	S/R-ratio
C_{max} (ng ml ⁻¹)	24.2 $\pm$ 8.2	32.0 $\pm$ 9.5*	21.0 $\pm$ 5.9	12.9 $\pm$ 5.2 [†]	1.73 {1.41–2.04}
t_{max} (h)	1.3 (0.5–3.0)	1.0 (0.5–3.0)	1.5 (1.0–3.0)	1.0 (0.5–1.50)	
AUC ^a (ng ml ⁻¹ h)	165 $\pm$ 60	186 $\pm$ 60	127 $\pm$ 42	69 $\pm$ 27 [†]	1.95 {1.55–2.28}
$t_{1/2,z}$ (h)	10.7 $\pm$ 9.4	10.9 $\pm$ 6.2	13.3 $\pm$ 8.6	13.5 $\pm$ 9.0	0.88 {0.63–1.18}
	<i>rac</i> - M_{II}	<i>rac</i> - M_{II}	<i>S</i> - M_{II}	<i>R</i> - M_{II}	
C_{max} (ng ml ⁻¹)	29.9 $\pm$ 9.8	42.7 $\pm$ 11.4*	8.9 $\pm$ 2.5	35.0 $\pm$ 11.1 [†]	0.28 {0.19–0.37}
t_{max} (h)	2.0 (1.0–4.0)	2.0 (1.0–3.0)	1.5 (1.0–2.0)	2.0 (1.0–3.0)	
AUC [‡] (ng ml ⁻¹ h)	269 $\pm$ 131	292 $\pm$ 92	57 $\pm$ 17	238 $\pm$ 89 [†]	0.26 {0.17–0.35}
$t_{1/2,z}$ (h)	10.1 $\pm$ 4.9	10.9 $\pm$ 4.7	12.5 $\pm$ 9.1	9.8 $\pm$ 4.9	1.09 {0.68–2.00}
CL_R (ml min ⁻¹)	3.39 $\pm$ 1.29	3.60 $\pm$ 2.17	3.15 $\pm$ 1.92	4.58 $\pm$ 2.66	0.71 {0.58–0.78}
Ae (% Dose)					
Free	0.52 $\pm$ 0.28	0.87 $\pm$ 0.61*	0.14 $\pm$ 0.08	0.90 $\pm$ 0.65 [†]	0.17 {0.11–0.23}
Conjugated	11.18 $\pm$ 4.86	17.0 $\pm$ 6.2*	13.2 $\pm$ 4.7	3.5 $\pm$ 1.8 [†]	4.24 {3.26–5.23}

ND = not determined.

^aAUC values were 0– ∞ for single dose and 0–12 h at steady-state.

[□]mean $\pm$ s.d. {95% CI}, except median (range) for t_{max} .

*Significantly different from the same parameter after single dose.

[†]R is significantly different from S.

Figure 2 Mean steady-state serum concentrations of *R*- (open symbols) and *S*-enantiomers (closed symbols) of pazinaclone (a) and M_{II} (b) after twice daily oral administration of 4 mg (triangles) and 8 mg (squares) *rac*-pazinaclone ($n = 9$).

and AUC values of *S*-pazinaclone were 71% (95% CI: 35%–104%) and 92% (95% CI: 55%–132%), respectively, higher than those of *R*-pazinaclone. No significant difference was found between the mean $t_{1/2,z}$ values for *S*- and *R*-pazinaclone. Regardless of drug dose, mean steady-state C_{max} and AUC values for *R*- M_{II} were about 3-fold higher than those of *S*- M_{II} (95% CI: 206%–417% and 224%–451% for C_{max} and AUC, respectively). However, within each dose group, the terminal half-lives of *R*- and *S*- M_{II} were similar and each was not significantly different from the $t_{1/2,z}$ of the respective enantiomer of pazinaclone. Renal clearances of unconjugated *R*- and *S*- M_{II} were similar but urinary recovery of the conjugated form was almost five times greater for *S*- M_{II} (95% CI: 370%–680%).

The fraction of unbound *rac*-pazinaclone was concentration-independent, ranging between 0.057 ± 0.005 and 0.064 ± 0.007 for concentrations up to 500 ng ml^{-1} . At 20 ng ml^{-1} the fraction of unbound *S*-pazinaclone ($0.045 \pm 0.001 \text{ s.d.}$) was 30% lower than that of *rac*-pazinaclone ($0.064 \pm 0.07 \text{ s.d.}$; 95% CI: 11%–49%). The binding of *S*-pazinaclone was not affected by the presence of *R*-pazinaclone at an *S/R*-ratio of 2:1 ($0.044 \pm 0.008 \text{ s.d.}$) or at the 1:1 ratio of the racemic mixture ($0.043 \pm 0.012 \text{ s.d.}$). Additionally, the fraction of unbound *S*-pazinaclone was not affected by the presence of *R*- and *S*- M_{II} in a 4:1 ratio. The fractions of unbound *rac*- and *S*- M_{II} were also concentration-independent, averaging 0.093 ± 0.011 and 0.080 ± 0.006 , respectively. The estimated f_u for *R*- M_{II} was slightly higher than that of the antipode, averaging 0.107 ± 0.024 . Blood/plasma

ratios of 0.72 ± 0.03 , 0.82 ± 0.040 , and 0.62 ± 0.05 were observed for *rac*-, *S*-, and *R*-pazinaclone, respectively, and were independent of drug concentration.

Discussion

The results suggest that the kinetics of *rac*-pazinaclone and M_{II} and their enantiomers are linear over the dose range studied. However, there was some evidence of a diurnal variation as indicated by differences in steady-state morning and evening mean C_{min} values and their *S/R*-ratios. This may reflect a decrease in gastrointestinal motility or prolonged gastric retention after the evening dose [9].

The *ex vivo* fractions of unbound *S*- and *R*-pazinaclone were not determined in the serum samples collected in the present study. However, the *in vitro* mean unbound fraction inferred for *R*-pazinaclone in human plasma was 1.84-fold higher than that measured for *S*-pazinaclone. Based upon this difference, AUC values of the unbound isomers were similar (e.g., 8 mg dose: 5.71 vs $5.73 \text{ ng ml}^{-1} \text{ h}$). Therefore, stereoselectivity in their kinetics appears to reside largely in plasma binding differences and is unrelated to differences in intrinsic clearance.

The intermediate M_I metabolite was not detectable in serum or urine samples, suggesting that it is rapidly reduced by the liver to M_{II} . Since data obtained using human liver slices (unpublished) indicate exclusive conversion of both *S*- and *R*-pazinaclone to the M_{II} metabolite, the fraction of the absorbed dose of pazinaclone converted to M_{II} appears to be unity for each enantiomer. Therefore, this excludes the possibility of stereoselectivity in the extent of metabolite formation after oral administration of the racemate. Thus, the four-fold higher AUC for *R*- M_{II} should reflect stereoselectivity in protein binding and/or subsequent metabolism of M_{II} . Since the unbound fraction of *S*- M_{II} is only slightly higher than that of the *R*-isomer, it seems that the subsequent stereoselective glucuronidation of M_{II} is the principal determinant of the four-fold higher AUC for *R*-pazinaclone. Although the renal clearances of the free *S*- and *R*-enantiomers of M_{II} were similar, the four-fold higher urinary recovery of conjugated *S*- M_{II} indicates that this enantiomer is a better substrate for glucuronyl transferase, which was reflected in the AUC differences. Serum concentrations of the metabolite declined in parallel to those of parent drug, resulting in comparable terminal half-lives for the *S*- and *R*-enantiomers of pazinaclone and M_{II} . This indicates that elimination of the metabolites is rate-limited by their formation.

In conclusion, oral pazinaclone was well tolerated in healthy human subjects. Stereoselectivity was found in the intrinsic clearance of the M_{II} metabolite, but not for parent pazinaclone. For parent, enantiomeric differences in total serum concentrations reflect differences in protein binding. Since only the *S*-enantiomers of pazinaclone and M_{II} bind to the benzodiazepine receptor, further measurements of the effects of the drug in patients should be related to the plasma concentrations of the *S*-enantiomers of parent and M_{II} .

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