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THE PHARMACOKINETICS OF ENDRALAZINE IN ESSENTIAL HYPERTENSIVES AND IN NORMOTENSIVE SUBJECTS

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1 The direct-acting vasodilator, endralazine, in combination with a β-adrenoceptor blocker significantly reduced the blood pressures of normotensive volunteers and of patients with essential hypertension.

2 The mean terminal elimination half-life for endralazine of 136 min in hypertensive patients did not differ significantly from the 155 min in normotensive subjects.

3 In normal subjects the mean oral bioavailability for endralazine was 75% and the mean clearance was 780 ml/min.

4 There were no significant pharmacokinetic differences between fast and slow acetylators.

5 The administration of endralazine to steady state in the hypertensive patients was associated with an increase in terminal elimination half-life and a decrease in the rate of absorption. However, there was no accumulation of endralazine with chronic dosing.

Keywords endralazine pharmacokinetics essential hypertension acetylator status

Introduction

Endralazine (6-benzoyl-3-hydrazino-5,6,7,8-tetra hydropyrido (4,3-C) pyrodazine mesylate) is a direct acting vasodilator structurally similar to hydralazine (Figure 1). In combination with a β-adrenoceptor blocker and a diuretic, endralazine is an effective

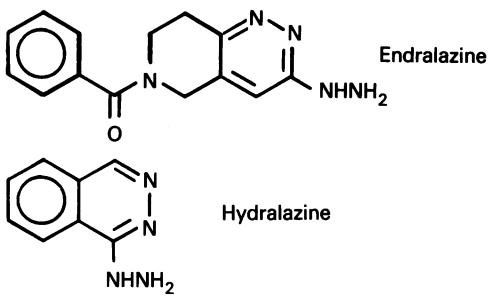

Figure 1 The structures of endralazine and hydralazine.

antihypertensive agent (Elliott *et al.*, 1982, 1983; Kirch & Axthelm, 1982). Although endralazine is structurally similar to hydralazine it appears that acetylation is not a major pathway for metabolism of the drug (Reece *et al.*, 1981a) and it thus has been claimed that endralazine may be less likely to cause immunological side effects, particularly the drug-induced lupus erythematosus syndrome. At present the metabolic and pharmacokinetic data for endralazine in man are limited to a small single dose study (Reece *et al.*, 1981a) which suggests that the parent drug can be measured in plasma after oral dosing and is eliminated with a half-life of 2 to 3 h. It was further demonstrated in this study that *in vitro* the rate of hydrazone formation with endogenous pyruvate and (+)-ketoglutaric acid in plasma was much less rapid for endralazine than for hydralazine.

The present study was undertaken to investigate the clinical pharmacokinetics of endralazine after oral and intravenous dosing in normotensive subjects and with single and multiple doses in patients with essential hypertension. All subjects and patients gave written informed consent and the study protocol was reviewed and approved by the local Research and Ethical Committee.

Methods

Patient study

Eight patients (five males, three females: aged 49–66 years) with essential hypertension and normal renal function whose blood pressures were not satisfactorily controlled on standard doses of a β-adrenoceptor blocker and diuretic were studied. The doses of β-adrenoceptor blocker and diuretic were continued unchanged for the duration of the study.

On study day 1 all patients received 10 mg endralazine orally and blood samples were collected before dosing and at 10, 20, 30, 45 min and at 1, 1.5, 2, 2.5, 3,

4, 5, 6, 7, 8 and 10 h after dosing for assay of plasma endralazine. Blood pressure and heart rate, supine and erect, were measured at hourly intervals by semi-automatic sphygmomanometer (Roche Arteriosonde 1225) and by radial pulse counts. Thereafter, the patients were established on a dose regimen of 10 mg endralazine twice daily (five patients) or 5 mg twice daily (three patients) depending upon their response to the initial dose. After 7 days of endralazine treatment the study was repeated (study day 2) with measurements at the same time intervals following the supervised administration of the morning dosage, 5 or 10 mg as appropriate. Thereafter, the endralazine dosage was adjusted as necessary aiming for blood pressure control at 140/90 mm Hg and the patients were reviewed every 2 weeks as out-patients.

After not less than 4 weeks therapy trough concentrations of endralazine were measured in a single plasma sample and after a further period of not less than 3 months of endralazine therapy a final study with the same protocol used on days 1 and 2 was carried out in four of the eight subjects (study day 3).

Normotensive subjects

Six normal healthy males (aged 24–35 years and weight 65–79 kg) with supine blood pressures less than 140/90 mm Hg were studied. The subjects were studied on two occasions at least 1 week apart and in random order received either 10 mg endralazine orally or 2 mg intravenously. Thirty minutes prior to both intravenous and oral endralazine administration the subjects received a single oral dose of 100 mg atenolol (β_1-selective adrenoceptor antagonist) to attenuate the reflex tachycardia associated with vasodilatation. Following intravenous administration blood samples were withdrawn at 0, 1, 2, 5, 10, 15, 20, 30, 45 min and at 1, 1.5, 2, 3, 4, 5, 6, 7 and 8 h after the end of the injection, whilst after oral administration blood samples were withdrawn for drug concentration analysis at the time detailed in the protocol for the essential hypertensives. Blood pressure and heart rate were measured at hourly intervals.

Endralazine assay

Plasma concentrations of endralazine were determined by the high performance liquid chromatographic assay described by Reece *et al.* (1981b). The assay is dependent upon the formation of a fluorescent derivative of the parent drug by heating with formic acid. In the study of hypertensive patients plasma samples were derivatised 'on line' and corresponding underivatised plasma samples were also studied after storage at −70°C. Samples in the concentration ranges 20–50, 50–100 and 100–500 nmol/l (six per concentration range) were stored for 7 and 12 days and then assayed (results of this are shown in Table 1) and, in the light of the results obtained, it was considered satisfactory in the normal volunteer bioavailability study to store the underivatised samples and analyse within 1 week of collection. The limit of detection of the assay, defined arbitrarily as three times baseline noise, was 5 nmol/l. Intra-assay reproducibility was determined by assaying replicate plasma samples containing added amounts of drug at various concentrations in the standard curve range of 10–500 nmol/l and the mean coefficient of variation over this range was 6.9%. There was no interference in the assay of endralazine by atenolol, bendrofluazide, hydrochlorthiazide and the pyruvate conjugate of endralazine.

Table 1 The stability of underivatised plasma samples stored at −70°C for 7 and 12 days. Samples in the concentration ranges 20–50, 50–100 and 100–500 nmol/l (six per concentration range) were stored for 7 and 12 days and then assayed.

Endralazine concentration range (nmol/l)	*7 days storage (% of day 0 concentration)*	*12 days storage (% of day 0 concentration)*
100–500	98 ± 4	96 ± 6
50–100	101 ± 5	91 ± 8
20–50	97 ± 6	89 ± 9

Acetylator status

Acetylator status was determined by the method of Carr *et al.* (1978). After fasting overnight subjects received a single oral dose of dapsone (100 mg) and exactly 3 h later a venous blood sample was collected for h.p.l.c. analysis of dapsone and monoacetyl dapsone. Phenotyping was based on the 3 h ratio of monoacetyl dapsone to dapsone: a ratio of 0.3, or greater, was interpreted as representing a fast acetylator and a ratio less than 0.3 as representative of a slow acetylator.

Pharmacokinetics

The pharmacokinetic profiles of endralazine were evaluated by computer assisted least squares fitting. In all subjects in both hypertensive and normotensive studies the data were most appropriately fitted by a two-compartment model. With oral endralazine, in the hypertensive patient study on day 1 and in the normal volunteer oral study, this model was described by the following equation:

$$Cp(t) = Ae^{-\alpha(t - t_{lag})} + Be^{-\beta(t - t_{lag})} - (A + B)\,e^{-ka(t - t_{lag})}$$

In the hypertensive patient study on the repeat

days, 2 and 3, the two-compartment model was described by the following equation:

$$Cp(t) = Ae^{-\alpha(t - t_{lag})} + Be^{-\beta(t - t_{lag})} - (A + B)e^{-ka(t - t_{lag})} + Coe^{-\beta(t)}$$

where Co = trough concentration of endralazine at time zero.

The disposition of intravenous endralazine was described by the following equation:

$$Cp(t) = Ae^{-\alpha(t)} + Be^{-\beta(t)}$$

Statistical analysis

Paired *t*-tests were used in the comparison of pharmacokinetic parameters on different study days in the patient study. The remaining statistical analyses were by unpaired Student's *t*-test and Wilcoxon rank test.

Results

Patients with essential hypertension

The parameter values obtained by fitting the plasma concentration data to the appropriate kinetic models on study days 1, 2 and 3 are shown in Table 2, whilst Table 3 shows the figures derived from these fitted parameters. Representative drug concentration-time profiles for each of the three study days are shown in Figure 2. It is apparent that the kinetics of endralazine at steady state (days 2 and 3) differ from those obtained at first dosing (day 1). The terminal elimination (β) half-life of endralazine was significantly longer ($P < 0.001$) in all subjects on study day 2 with a mean of 448 ± 236 min, compared with 136 ± 49 min for day 1. There was a similar pattern for the absorption rate constant (ka): the absorption half-life being significantly ($P < 0.001$) longer in all subjects with a mean of 16.7 ± 10.7 min on study day 2, compared to a mean of 3.7 ± 3.7 min on study day 1. There was also a significant reduction ($P < 0.001$) in peak drug concentrations (corrected where appropriate for a change in endralazine dosage) on day 2 (mean 245 ± 170 nmol/l) when compared to day 1 (512 ± 250 nmol/l). The changes in pharmacokinetics were not related to the dose regimen established for maintenance therapy. Despite these alterations in pharmacokinetic parameters the areas under the concentration time curves (corrected for dosage) were not significantly different on days 1 and 2. In the four patients whose pharmacokinetic study was repeated for a third study day following at least 3 months' therapy, there was no significant difference in the disposition of endralazine on that day (day 3) compared to the disposition after 7 days' therapy (day 2). The variability in the parameter values derived on days 2 and 3 (Table 3) reflect the errors inherent in determining relatively long half-lives from a 10 h sampling period.

Table 4 compares the endralazine trough concentrations following at least 4 weeks' therapy with the trough concentrations predicted from both the kinetic

Table 2 Fitted pharmacokinetic parameters of oral endralazine on first dosing (day 1) and a steady state (days 2 + 3) in hypertensive patients.

Patient	*Day*	*Dose (mg)*	*A (nmol/l)*	*α (min⁻¹)*	*B (nmol/l)*	*β (min⁻¹)*	*ka (min⁻¹)*	t_{lag} *(min)*	Co *(nmol/l)*
1	1	10	674	0.021	77	0.0038	1.44	20	—
	2	10	6471	0.017	42	0.00089	0.019	26	52
	3	10	788	0.024	38	0.0015	0.057	15	21
2	1	10	508	0.025	148	0.0056	0.35	19	—
	2	5	2045	0.097	48	0.0049	0.12	20	7
	3	5	755	0.028	40	0.0040	0.099	29	5
3	1	10	678	0.023	46	0.0031	0.10	9	—
	2	10	273	0.019	60	0.0026	0.04	10	7
4	1	10	1188	0.030	169	0.0058	0.25	45	—
	2	5	553	0.026	33	0.0022	0.088	42	17
5	1	10	2970	0.19	421	0.011	0.40	20	—
	2	10	691	0.019	27	0.0014	0.11	20	50
	3	10	374	0.014	12	0.0013	0.34	28	39
6	1	10	174	0.027	114	0.0065	0.38	10	—
	2	5	349	0.023	22	0.00087	0.05	43	28
	3	5	347	0.013	18	0.00071	0.02	25	20
7	1	10	351	0.023	76	0.0047	0.35	44	—
	2	10	1744	0.022	34	0.0020	0.026	17	19
8	1	10	1534	0.039	103	0.0057	0.059	10	—
	2	10	2697	0.040	26	0.0016	0.043	43	28

Table 3 Derived parameters for oral endralazine at first dosing (day 1) and a steady state (days 2 and 3) in hypertensive patients.

Patient	*Day*	*Dose (mg)*	$t_{1/2}, \alpha$ *(min)*	$t_{1/2}, \beta$ *(min)*	$t_{1/2}, ka$ *(min)*	*Area under curve (nmol l^{-1} min)*	*Peak concentration (nmol/l)*	*Time to peak (min)*
1	1	10	33	182	0.5	51800	753	30
	2	10	41	779	36.5	85200	409	45
	3	10	29	442	12.0	42566	286	45
2	1	10	28	124	2.0	44900	509	30
	2	5	7	141	5.8	13400	201	30
	3	5	24	174	0.7	44645	548	45
3	1	10	30	223	7.0	36900	380	30
	2	10	37	267	17.0	29200	112	45
4	1	10	23	120	2.8	63300	877	60
	2	5	27	315	7.9	29700	256	60
5	1	10	4	63	1.7	45400	753	30
	2	10	37	495	6.3	48800	410	45
	3	10	49	517	2.1	33844	315	45
6	1	10	26	107	1.8	23200	232	20
	2	5	30	797	17.3	33638	114	90
	3	5	54	972	30.4	36108	100	90
7	1	10	30	147	2.0	30200	313	60
	2	10	32	347	26.7	27900	118	60
8	1	10	18	122	11.7	29600	280	45
	2	10	17	443	16.1	20300	88	60

parameters determined on day 1 and the kinetic parameters determined on day 2. The measured concentrations were far more closely correlated to the estimates predicted from the day 2 kinetics ($r = 0.93$, $P < 0.001$) than the concentrations predicted from day 1 kinetics ($r = -0.22$, NS).

Normotensive subjects

Typical kinetic profiles following intravenous and oral endralazine administration are shown in Figure 3

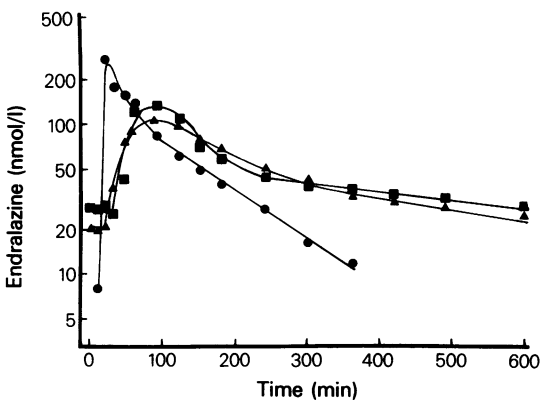

Figure 2 Representative (patient 6) oral drug concentration-time profiles on first dosing (day 1 ●——●), following 1 week's therapy (day 2 ▲——▲) and after at least 3 months' therapy (day 3 ■——■).

and the results of the pharmacokinetic analyses of the intravenous and oral plasma concentration data are given in Tables 5 and 6 respectively. Table 7 shows the figures derived from these fitted parameters. In five of the six normal volunteers the terminal elimination (β) half-life following oral dosing was longer than that following intravenous dosing but there was no statistically significant difference from the group results for oral and intravenous administration with mean terminal elimination (β) half-lives of 155 ± 30 min and 111 ± 47 min respectively. The clearance of intravenous endralazine ranged from 462 to 1151 ml/min with a mean of 778 ml/min and oral bioavailability ranged from 43 to 97% with a mean of 74.5%.

Acetylator status

In the hypertensive study patients 1, 2, 3, 4 and 6 were classified as slow acetylators whilst patients 5, 7 and 8 were classified as fast acetylators. Normotensive subjects 2, 5 and 6 were found to be slow acetylators and subjects 1, 3 and 4 were fast acetylators.

As there were no significant differences between normotensives and hypertensives following a single oral dose of endralazine and with the small numbers in each group, the data from fast and slow acetylators were pooled. No significant differences were observed between the fast and the slow acetylators for both terminal elimination (β) half-life and area under the concentration-time curve (AUC) for parent drug (Table 8). No acetylated metabolite was detected in

Table 4 Trough concentrations of endralazine measured after at least 4 weeks dosing compared with concentration predicted from day 1 and day 2 kinetics.

Patient	*Endralazine concentration measured > 4 weeks dosing (nmol/l)*	*Predicted concentration of endralazine based on day 1 and day 2 kinetics (nmol/l)*	*(nmol/l)*
1	16	5.3	15.3
2	8	1.4	1.8
3	10	5.5	10.9
4	10	1.3	8.6
5	16	0.15	15.3
6	20	0.65	25.7
7	12	2.7	10.6
8	15	1.7	12.0

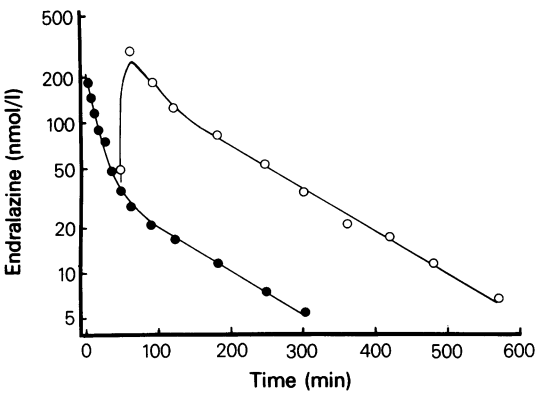

Figure 3 Representative (subject 4) drug concentration-time profiles following intravenous (●——●) and oral (O——O) endralazine.

Table 5 Fitted pharmacokinetic parameters of intravenous endralazine (2 mg) in normotensive subjects.

Subject	*A (nmol/l)*	*α (min⁻¹)*	*B (nmol/l)*	*β (min⁻¹)*
1	219	0.173	64	0.0073
2	109	0.090	40	0.0034
3	176	0.095	34	0.0078
4	175	0.075	38	0.0065
5	303	0.116	44	0.0081
6	175	0.126	31	0.0080

any of the studies, irrespective of the route of drug administration or the dosage frequency.

Discussion

It is apparent from this study that the pharmacokinetics of endralazine are different from those of hydralazine to which it is structurally related. In both normal volunteers and hypertensive patients, on first oral dosing, the terminal elimination half-life of endralazine of 2–3 h was considerably longer than the 40–60 min reported for hydralazine (Shepherd *et al.*, 1980; Reece *et al.*, 1980b). It is also clear that, unlike hydralazine, there are no significant differences in the pharmacokinetic profiles of endralazine in fast and slow acetylators. The mean area under the concentration-time curve (AUC) following oral endralazine was apparently larger in slow acetylators than in fast acetylators but this was not statistically significant.

The oral bioavailability of endralazine in normal volunteers was high, about 75% on average, indicating that the drug was not subject to extensive first-pass metabolism and there was no evidence that the oral bioavailability of endralazine was higher in slow acetylators than in fast acetylators. Overall, these findings provide indirect evidence that there is no extensive hepatic acetylation of endralazine, in contrast to hydralazine which has been shown to have large and significant differences in AUC and bioavailability between the two acetylator phenotypes (Reece *et al.*, 1980; Shepherd *et al.*, 1980). This is further supported

Table 6 Fitted pharmacokinetic parameters of oral endralazine (10 mg) in normotensive subjects.

Subject	*A (nmol/l)*	*α (min⁻¹)*	*B (nmol/l)*	*β (min⁻¹)*	*ka (min⁻¹)*	*t_{lag} (min)*
1	543	0.035	74	0.0037	0.42	19.6
2	648	0.056	80	0.0045	0.55	9.1
3	95	0.043	86	0.0040	0.13	43.0
4	202	0.019	142	0.0057	0.34	44.5
5	758	0.042	86	0.0038	0.46	17.4
6	550	0.047	60	0.0055	1.42	10.2

Table 7 Derived parameters for intravenous and oral endralazine in normotensive subjects.

Subject		$t_{1/2}\beta$ *(min)*	*Area under curve (nmoll^{-1}min)*	*Clearance (ml/min)*	*Bioavailability (%)*
1	Oral	187	32452		65
	i.v.	94.4	10033	602	
2	Oral	153	27812		43
	i.v.	206	12993	462	
3	Oral	172	22324		72
	i.v.	89.2	6183	970	
4	Oral	122	34746		85
	i.v.	107	8136	736	
5	Oral	181	38661		97
	i.v.	85.5	7989	751	
6	Oral	117	22088		85
	i.v.	86.2	5212	1151	

Table 8 The mean terminal elimination (β) half life of endralazine and mean area under concentration-time curve in fast and slow acetylators in combined groups of hypertensive patients and normotensive subjects on first dosing.

	$t_{1/2}\beta$ *(min)*	*Area under curve (nmol l^{-1} min)*
Slow acetylators $n = 8$	151 ± 42	38600 ± 14400
Fast acetylators $n = 6$	135 ± 44	32450 ± 7600

by the fact that although it is possible to detect acetylated metabolite by the assay system used in the present study (Reece *et al.*, 1981b) none was detected in the plasma samples following both single and multiple doses of endralazine.

A further difference between hydralazine and endralazine was observed in the steady state kinetics. It has been shown that the disposition of hydralazine is the same following first and chronic dosing (Ludden *et al.*, 1982) whereas the pharmacokinetics of endralazine at steady state were different from those at first dosing, with a prolongation of terminal elimination half-life and increased absorption rate half-life. The precise explanation for this alteration in kinetics is unclear but despite these changes in drug kinetics, there was no evidence of endralazine accumulation on chronic therapy, either at 1 week, 4 weeks, or 3 months.

In conclusion, therefore, although the pharmacological actions of endralazine are similar to those of hydralazine (Elliott *et al.*, 1982; Kirch & Axthelm, 1982) the pharmacokinetic profile and routes of metabolism show significant differences which require further investigation to fully evaluate their clinical relevance.

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