Pharmacokinetics and biochemical efficacy of idrapril calcium, a novel ACE inhibitor, after multiple oral administration in humans

P. J. WYLD¹, J. GRANT¹, A. LIPPI², M. CRISCUOLI², G. DEL RE³ & A. SUBISSI²

¹Inveresk Clinical Research Ltd, Edinburgh, U.K., ²Laboratori Guidotti SpA, Pisa, Italy and ³A. Menarini Pharmaceuticals, Firenze, Italy

- 1 The pharmacokinetic profile and biochemical efficacy of idrapril calcium, a novel angiotensin converting enzyme (ACE) inhibitor, were evaluated in healthy volunteers after multiple dosing for 5 days at the doses of 100, 200 and 400 mg twice daily. The study was conducted as a double-blind, cross-over comparison of idrapril calcium against placebo.
- 2 Plasma concentrations of idrapril were determined by an indirect enzymatic method. Urinary concentrations were measured by reverse phase high performance liquid chromatography (h.p.l.c.). Plasma samples were also analysed for ACE activity.
- 3 The pharmacokinetics of idrapril calcium did not change significantly between day 1 and day 5. The values of $C_{\max}$ and AUC were dose-related over the range of doses tested; $t_{\max}$ was 3–4 h and apparent elimination half-life was 1.4–1.6 h.
- 4 Plasma ACE activity was maximally inhibited (94–96%) at all dose levels and remained more than 80% depressed from 2 to at least 6 h after idrapril calcium. Although the maximum effect was not dose-related, the duration of inhibition showed some dose-dependency, ACE activity returning to 56, 45 and 29% of the basal value 12 h after the 100, 200 and 400 mg doses, respectively.
- 5 There were no clinically significant adverse events experienced by the volunteers. No dose-related effects on blood pressure or heart rate were observed. There were no changes in clinical pathology tests, urine analyses or electrocardiograms after dosing with idrapril calcium.
- 6 Idrapril calcium, the prototype of a new class of ACE inhibitors, appears to be well-tolerated. Its pharmacokinetics were not significantly changed after repeated administration in man. Plasma ACE activity was markedly depressed for more than 12 h even after the lowest dose tested (100 mg) and inhibition was correlated to the levels of circulating drug, the *ex vivo* IC_{50} being practically identical (12 ng ml^{-1}) on day 1 and day 5.

Keywords ACE inhibitor idrapril calcium pharmacokinetics human volunteers

Introduction

Angiotensin converting enzyme (ACE) inhibitors have been used successfully to treat hypertension and congestive heart failure for many years. The ACE inhibitors currently marketed are dipeptide or aminoacid derivatives that differ mainly in the chemical nature of their zinc binding ligand. Idrapril cal-

cium belongs to a recently discovered class of ACE inhibitors, namely the hydroxamic non-aminoacid derivatives [1]. Idrapril calcium differs from traditional ACE inhibitors in that the amide bond is shifted with the consequence that it does not contain a cyclic aminoacid such as proline or substituted pro-

line. Furthermore the zinc binding ligand of idrapril calcium is a hydroxamic group instead of the sulphhydryl group of captopril or the carboxyl group of enalapril and its analogues or the phosphinyl group of fosinopril. In biological terms, idrapril competitively inhibits rabbit lung ACE with a K_i of 0.5 nM, showing the characteristics of a slow and tight inhibitor [2], and retains the pharmacological properties of known ACE inhibitors *in vitro* and *in vivo* [3]. Idrapril is approximately as potent as captopril, but with a longer duration of action, and experimental results obtained in guinea-pigs (capsaicin-induced bronchoconstriction) suggest that it may be less liable to produce cough in patients [3].

In healthy volunteers intravenous idrapril (1 to 25 mg) dose-dependently modifies circulating renin angiotensin system (active renin, angiotensin I, angiotensin II, angiotensin II/angiotensin I ratio, ACE) as expected by an ACE inhibitor [4]. Idrapril calcium, a stable salt of idrapril, reduces plasma ACE activity after oral administration of 100 mg and has a bioavailability of approximately 30% [4, 5].

The pharmacokinetic profile of single oral doses (100, 200 and 400 mg) of idrapril calcium in healthy volunteers has been reported, showing that C_{max} and AUC are dose related, t_{max} is 2 h and elimination half-lives are 2.1–2.5 h [6].

The objective of the present study was to investigate tolerability, pharmacokinetics and biochemical efficacy of idrapril calcium after multiple oral administration to healthy male volunteers.

Methods

Subjects

Six subjects, age 29.5 ± 11.0 years (mean value $\pm$ s.d.), body weight 70.6 ± 10.7 kg, were selected from a panel of healthy volunteers held at Inveresk Clinical Research (ICR), Edinburgh. All subjects had a medical history taken, satisfied a physical examination and laboratory evaluation and had a body weight within $\pm 15\%$ of their ideal. Subjects were excluded if they had taken an investigational drug in the 4 months before the study or if they needed any medication within 5 days of study entry. Additionally, those subjects who had lost greater than 400 ml of blood in the 3 month period before study entry, were excluded. All subjects gave their written informed consent to participate in the study which was approved by the Ethics Review Committee of ICR. The study was conducted as double-blind, cross-over comparison of idrapril calcium against placebo. The subjects were randomized to receive placebo or idrapril calcium 100, 200 or 400 mg. Doses were administered twice daily for 5 days. Randomization was forced so that idrapril calcium was given to subjects in ascending order.

Drugs

Idrapril calcium ((+)-(1S,2R)-2-{[N-(2-hydroxyamino-2-oxoethyl)-N-methyl amino]carbonyl}cyclohexane-1-

carboxylic acid, calcium salt) was supplied by Laboratori Guidotti (Pisa, Italy). Active drug and placebo were administered in hard gelatin capsules. Doses were always expressed in terms of idrapril free acid.

Study design

Morning dosing was preceded by an overnight fast and a standard meal was given immediately after dosing. Morning doses were given at intervals from 08.30 h and evening doses from 20.30 h, so that there was a 12 h interval between each subject's doses.

On all study days subjects remained recumbent for 2 h after dosing, after which time they were permitted to be ambulant.

Blood samples were taken for haematology and clinical chemistry at screening, before morning dose on day 1 and 3, and 36 h after the last dose. ECG was performed at screening, before morning dose on each treatment and 6 h after the morning dose on day 1 and 3. Vital signs (supine systolic and diastolic blood pressure and heart rate) were measured at screening, before the morning dose on each treatment day, 2, 4, 6 and 12 h after the morning dose on day 1, 3 and 5, and 12 and 36 h after the last treatment. Urinalysis was performed using Multistix (Ames) on screening and 36 h after the last dose. Blood sampling for pharmacokinetic analysis and ACE activity was performed on a number of occasions before and after dosing (Figures 1 and 2). Urine collection periods were 0–12 h after the first administration and 0–12 h and 12–36 h after the last administration.

Subjects were resident during each dosing period from the evening before dosing until 36 h after the last dose. There was a minimum wash-out period of one week between different treatments.

Analytical procedures

Plasma concentrations Concentration of idrapril in plasma was determined by an enzymatic method, based on the inhibition of a standard preparation of purified ACE by extracts of plasma samples, as previously described [5]. The limit of quantitation of idrapril was 1 ng ml⁻¹.

Urine concentrations Concentration of idrapril in urine was determined by a h.p.l.c. method with electrochemical detection, as described elsewhere [7]. The limit of quantitation was 2.5 µg ml⁻¹.

Plasma ACE activity ACE activity in plasma samples was assayed by measuring the amount of hippuric acid cleaved from the substrate hippurylglycylglycine by h.p.l.c. with spectrophotometric detection, as described elsewhere [5].

Data analysis

Pharmacokinetic parameters The post-absorptive phase of the plasma concentration curves was treated as a monoexponential decay and the rate constant λ_z was obtained by the fitting utility of the CricketGraph

program (Cricket Software, PA, USA) on Macintosh computers. Elimination half-life ($t_{1/2}$) was calculated as $\ln 2/\lambda_z$ and the area under the curve (AUC) was computed by the trapezoid rule over the 0–12 h time interval after treatment.

Correlation between ACE activity and plasma concentrations The effect of idrapril on plasma ACE, in terms of activity relative to the predose value, was correlated with idrapril concentration through a four-parameter logistic equation [8] by using the MacALLFIT software (V. Guardabasso and G. Angeli, Consorzio Mario Negri Sud, Chieti Italy) for Macintosh computers.

Statistics Day 1 and day 5 data for $C_{\max}$ and AUC were compared by the Student's t -test. λ_z and $t_{1/2}$ values were submitted to one-way analysis of variance (InStat Mac by GraphPad Software, San Diego CA, USA). A value of $P < 0.05$ was taken as statistically significant.

Results

Tolerability, blood pressure and heart rate

A number of adverse events were recorded in each experimental group (Table 1). None of the adverse events, the most common one being light-headedness, was serious and the nature and frequency of the

Table 1 Adverse events following multiple dosing of healthy volunteers with ascending doses of idrapril calcium. Events are reported by system (Costart terminology)

System	Placebo	Multiple dose		
		100 mg	200 mg	400 mg
Body as a whole	3	1	5	1
Cardiovascular	1	0	0	0
Digestive	1	0	1	0
Neurological	0	2	1	1
Respiratory	1	0	1	0
Skin	1	1	0	0
Special senses	1	0	0	0
Total	8	4	8	2

Figure 1 Plasma drug concentration-time profile in healthy volunteers following repeated twice daily 100 (▲), 200 (■) or 400 (●) mg doses of idrapril calcium. Means $\pm$ s.e. mean are reported, $n = 6$.

events did not relate to the dose. The results of routine laboratory tests, urine analyses and electrocardiograms were not modified by idrapril calcium. Idrapril calcium did not show any effect on blood pressure at any of the dose levels studied, while there were episodes of erect tachycardia in all dose groups including placebo. None of them appeared to be dose related.

Pharmacokinetics

Figure 1 shows mean plasma concentration profiles of idrapril following repeated treatments with the different doses of idrapril calcium. Pharmacokinetic parameters obtained on days 1 and 5 are summarized in Table 2. $C_{\max}$ and AUC values were linearly dose-related on both days and, although a trend towards increasing mean values was observed at the end of the treatment period with 200 and 400 mg doses, these inter-day differences were not statistically significant. Plasma concentrations at 2 and 12 h post-dose, measured daily, were also dose-related and remained relatively constant throughout the dosing period. Residual idrapril concentrations at 12 h, i.e. immediately before the next treatment, ranged from 10–40 ng ml⁻¹. This did not have any significant effects on the kinetics of the ensuing dose. Idrapril

Table 2 Pharmacokinetic parameters of idrapril calcium in man after multiple oral administration for 5 days, twice daily. Means $\pm$ s.e. mean ($n = 6$) are reported.

Day of treatment	Pharmacokinetic parameter (units)	Dose		
		100 mg	200 mg	400 mg
1	$t_{\max}$ (h)	3 (2–3)*	3.5 (2–6)	4 (3–4)
	$C_{\max}$ (ng ml ⁻¹)	430 $\pm$ 102	601 $\pm$ 51	1519 $\pm$ 222
	λ_z (h ⁻¹)	0.4440 $\pm$ 0.0147	0.4818 $\pm$ 0.0133	0.4924 $\pm$ 0.0123
	$t_{1/2}$ (h)	1.57 $\pm$ 0.05	1.44 $\pm$ 0.04	1.41 $\pm$ 0.04
	AUC(0,12 h) (μ g ml ⁻¹ h)	1.71 $\pm$ 0.45	2.61 $\pm$ 0.18	6.48 $\pm$ 0.92
5	$t_{\max}$ (h)	3 (2–3)	3.5 (2–6)	3 (2–4)
	$C_{\max}$ (ng ml ⁻¹)	387 $\pm$ 52	793 $\pm$ 200	2383 $\pm$ 446
	λ_z (h ⁻¹)	0.4353 $\pm$ 0.0216	0.4463 $\pm$ 0.0096	0.4573 $\pm$ 0.0162
	$t_{1/2}$ (h)	1.61 $\pm$ 0.08	1.56 $\pm$ 0.03	1.53 $\pm$ 0.05
	AUC(0,12 h) (μ g ml ⁻¹ h)	1.56 $\pm$ 0.21	3.17 $\pm$ 0.75	9.66 $\pm$ 1.63

*median (range)

Table 3 Total urinary excretion of unchanged idrapril after multiple oral administration of idrapril calcium. Means $\pm$ s.e. mean are reported ($n = 6$)

Day	100 mg		Dose 200 mg		400 mg	
	mg	% dose	mg	% dose	mg	% dose
1	8.0 $\pm$ 2.9	8.0 $\pm$ 2.9	11.3 $\pm$ 1.5	5.6 $\pm$ 0.7	18.2 $\pm$ 4.0	4.5 $\pm$ 1.0
5	1.4 $\pm$ 1.1	1.4 $\pm$ 1.1	11.4 $\pm$ 2.6	5.7 $\pm$ 1.3	35.0 $\pm$ 4.2	8.7 $\pm$ 1.1

was not detectable in plasma 36 h after the last administration. Values of λ_z and the derived $t_{1/2}$ did not vary either with dose or with repeated treatment.

Unchanged idrapril was found in urine. As shown in Table 3, a high inter-subject (as well as intra-subject on different days) variability was observed in urinary excretion. A maximum recovery of about 8% was obtained with the 100 mg dose on day 1 and with the 400 mg dose on day 5. No idrapril was measurable in urine collected 12–36 h after the last treatment and 0–12 h data appears to be representative of overall elimination of unchanged drug in urine.

Biochemical efficacy

Plasma ACE activity in pre-dose samples ranged from 53–131 mu ml⁻¹ with some (CV < 20%) intra-subject variability. As shown in Figure 2, treatment with placebo did not affect ACE throughout the observation period. Idrapril calcium markedly affected ACE activity after every dose and peak inhibition (94 to 96%) at 3 to 4 h after treatment was similar with all three doses. Time to recovery towards normal activity was dose-related, inhibition values of 44 $\pm$ 5, 55 $\pm$ 6 and 71 $\pm$ 3% being found 12 h after the first treatment with 100, 200 and 400 mg, respectively. Repeated dosing did not influence the effects of idrapril calcium and almost identical inhibition curves were obtained after the ninth treatment on day 5. ACE activity had returned to basal levels by 36 h after the tenth administration.

Correlation between ACE activity and plasma concentrations

ACE inhibition was found to correlate with log plasma concentration of idrapril according to a sigmoidal function (Figure 3). Idrapril concentration capable of inducing 50% inhibition of plasma ACE was virtually identical on day 1 and on day 5: 11.4 $\pm$ 1.0 vs 12.1 $\pm$ 0.8 ng ml⁻¹.

Discussion

This study suggests that idrapril calcium is a well-tolerated drug in healthy volunteers administered with doses up to 400 mg twice daily for 5 days. This period of dosing did not result in adverse effects that could be related to treatment for incidence or intensity. Large long-term studies in hypertensive patients are required in order to evaluate fully tolerability of this novel compound in the management of hypertension.

Figure 2 Time profile of plasma ACE inhibition in healthy volunteers following repeated twice daily 100 (▲), 200 (■) or 400 (●) mg doses of idrapril calcium or placebo (◇). Means $\pm$ s.e. mean are reported, $n = 6$. **Figure 3** Relationship between plasma drug concentration and ACE activity at the beginning and at the end of the repeated oral treatment with 100–400 mg doses of idrapril calcium in healthy volunteers. Individual data points are reported.

Following oral administration of idrapril calcium, peak concentrations were reached within 3–4 h at any dose level and C_{max} and AUC were correlated to dose, while the terminal half-lives (1.4–1.6 h) were dose-independent. In previous single dose studies, terminal half-lives were 1.5 h (100 mg, [5]), 2.1–2.5 h (100–400 mg, [6]) and 2.9 h (100 mg, [4]). These differences can be explained by the difficulty of correctly estimating the terminal half-life with ACE inhibitors, known to have a complex, not single-expo-

nential elimination from plasma [9]. Indeed the above half-lives were estimated over different time spans: up to 12 h from treatment in the present study and up to 12, 24 or 36 h, respectively, in the previous studies [4–6].

Moreover, delayed absorption maxima and reduced peak concentrations and AUCs were observed in this study compared with the previous ones. A reasonable explanation for this discrepancy can be sought in the protocols of the different studies: when idrapril calcium was administered as a single dose [4–6], subjects remained fasting for at least 4 h after treatment, whereas in the present study they received a standard meal soon after drug administration. The possibility exists that food interferes with idrapril absorption and bioavailability, as reported for captopril [10, 11].

Idrapril was found to be eliminated by renal excretion, although in amounts that are below those expected after the estimated oral bioavailability of about 30% [4]. This might be related to reduced absorption of idrapril calcium in the present study, as discussed above. The possibility that an alternate metabolic route of elimination exists for this compound is currently being investigated.

Comparison of pharmacokinetic parameters on day 1 and day 5 of treatment suggests that idrapril cal-

cium does not have time dependent pharmacokinetics. Plasma concentrations at any dose were statistically indistinguishable throughout the study and no consistent signs of accumulation or altered disposition were observed.

At all tested dose levels idrapril calcium inhibited plasma ACE to approximately the same extent ($\geq 94\%$), while duration of action was dose-related. These biochemical effects were very similar on day 1 and day 5 and substantial inhibition appeared after 12 h at any dose level. Inhibition of plasma ACE and the antihypertensive effect of ACE inhibitors are not simply temporally related [9, 12]. In a recent study performed in patients with essential hypertension, a single oral dose of idrapril calcium (200 mg) effectively lowered supine and erect blood pressure up to 24 h, a time when plasma ACE had returned to normal [13].

In conclusion, idrapril calcium appears to be well-tolerated after oral administration of multiple doses up to 400 mg twice daily in healthy volunteers. Drug accumulation or tolerance does not appear to occur after repeated administration. The duration of plasma ACE inhibition exceeds 12 h even at the lowest dose tested (100 mg). The drug may represent a valuable class of once-daily antihypertensive ACE inhibitors.

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