Pharmacokinetics of eltoprazine in healthy male subjects after single dose oral and intravenous administration

MAIKEL RAGHOEBAR¹, MARIANNE MAK¹, ANTOINE COURNOT², MARCEL C. M. PISTORIUS¹, JAAP VAN HARTEN¹ & HENK ROSEBOOM¹

¹Duphar B.V., P.O. Box 900, 1380 DA Weesp, The Netherlands, and ²Therapharm Recherches, 59 Rue de Billancourt, 92100 Boulogne Billancourt, France

The kinetics, safety and tolerability of eltoprazine hydrochloride were studied in an open, cross-over, partially randomised design after single oral (8 mg) and intravenous (3 and 8 mg) doses to 12 healthy male subjects. After intravenous administration, the mean $t_{1/2}$ ranged from 7 to 9 h, the MRT was 11 h, CL was 487 ± 148 (3 mg dose) and 471 ± 56 (8 mg dose) $\text{ml kg}^{-1} \text{h}^{-1}$, while CL_R was 226 ± 124 (3 mg dose) and 189 ± 38 (8 mg dose) $\text{ml kg}^{-1} \text{h}^{-1}$. The V_{ss} was 3.3 ± 0.7 (3 mg dose) and 3.8 ± 0.5 (8 mg dose) l kg^{-1} . Cumulative renal excretion was 40%. The AUC and the cumulative urinary excretion were directly proportional to dose within the range of 3–8 mg. Values of t_{max} varied from 1 to 4 h after oral administration. The mean C_{max} value was 24 ng ml^{-1} after an oral dose of 8 mg. The plasma elimination half-life after oral administration was 9.8 ± 3.9 h. Absolute oral bioavailability was $110 \pm 32\%$. Dose-dependent somnolence was observed.

Keywords pharmacokinetics eltoprazine serenics

Introduction

Eltoprazine hydrochloride (DU 28853) (Figure 1) is a novel compound which belongs to a new class of psychotropic drugs known as serenics (Olivier *et al.*, 1986). In animal tests of aggressive behaviour serenics appear to be specific inhibitors of offensive behaviour, leaving social and defensive behaviour intact (Olivier *et al.*, 1986;

Olivier & Mos, 1986). This action is exerted without impairment of sensory or motor functions and without sedation, in contrast with the actions of neuroleptics and other drugs used to suppress pathological destructive behaviour. The effects of serenics on aggression in animal experiments appear to be related to serotonergic (5-HT_{1A} and 5-HT_{1B}) activity (Bevan *et al.*, 1990; Olivier *et al.*, 1987, 1990). Eltoprazine hydrochloride (hereinafter called eltoprazine) appears to be a mixed 5-HT_{1A/1B} agonist, which exerts specific anti-aggressive effects in several aggression paradigms in animals (Bevan *et al.*, 1990; Olivier *et al.*, 1987). The present study was designed to determine the kinetics of eltoprazine, after single doses of 3 and 8 mg to 12 healthy male volunteers by the intravenous and oral routes. In addition, the safety and tolerability of the drug was monitored.

Figure 1 Chemical structure of eltoprazine hydrochloride [1-(2,3-dihydro-1,4-benzodioxin-5-yl)piperazine hydrochloride].

Correspondence: Dr Maikel Raghoobar, Department of Drug Disposition, Duphar B.V., P.O. Box 900, 1380 DA Weesp, The Netherlands

Methods

Subjects

Twelve healthy male subjects (aged 18 to 45 years) volunteered to participate in the study, the protocol of which was approved by the Therapharm Recherches Ethics Committee. They were all in good health, as judged by appropriate biochemical tests and physical examination, and had no evidence of haematologic, renal or hepatic dysfunction. The use of any drug (including over the counter drugs) within 2 weeks prior to the beginning of the study period and up to 72 h after drug intake was not allowed. No alcohol was permitted within 48 h prior to drug intake nor for 72 h after drug administration.

Study protocol

Each subject completed three 3-day study periods during which either 3 or 8 mg of the drug was administered intravenously or 8 mg was administered orally. In the first session of the study all volunteers received an intravenous infusion of 3 mg eltoprazine in 500 ml of saline over 1 h. The reason for starting with this low dose was the potential for adverse reactions. Therefore, a balanced study design was forgone in order to be assured that the volunteers would tolerate the lowest intravenous dose. Sessions two and three were performed according to a randomised cross-over design in blocks of six for each session. In these sessions the volunteers were given either 8 mg eltoprazine orally or 8 mg by intravenous infusion in 500 ml saline over 1 h. In the oral session the subjects took capsules containing 8 mg eltoprazine with 250 ml of water, over 2 min. One hour after dosing the volunteers in the oral session swallowed a further 250 ml water. During the first 4 h after drug intake all volunteers in all three sessions had a water intake of 500 ml. Treatment periods were separated by at least a 2-week washout interval. For the study sessions, the subjects were institutionalised in the evening prior to the day of drug intake, and for 24 h after drug intake. Pre- and post-treatment assessments included clinical examination, 12-lead ECG, safety laboratory assessments, urine drug screen, vital signs (heart rate and blood pressure, measured after 10 min of supine rest), spontaneous reporting of signs and symptoms, and the collection of blood and urine samples. Subjects remained fasting for a minimum of 10 h before dosing.

One hour before dosing, five biscuits and 200 ml of fruit juice were consumed. Thereafter no food was permitted until 4 h after drug admin-

istration, when lunch was provided. In the evening a meal was served. Caffeine containing drinks were not allowed on the study days. Blood samples (10 ml) were collected from a forearm vein at the following times: 0 (immediately before eltoprazine administration), 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, 24, 48 and 72 h after dosing. Urine was collected over the intervals: 0–1.5, 1.5–3, 3–4, 4–5, 5–6, 6–7, 7–9, 9–11, 11–13, 13–16, 16–24, 24–48 and 48–72 h after dosing. Blood samples were collected in heparinized tubes and centrifuged cold within 0.5 h of sampling. Plasma samples and aliquots of the urine collections were stored at -20°C until analysis.

Reagents

Dansyl chloride was obtained from Pierce (Rockford, Illinois, USA). DU 121334 (1-(1,3-benzodioxol-4-yl) piperazine hydrochloride) was used as internal standard in the assay of eltoprazine (synthesized by Duphar B.V., Weesp, The Netherlands). All other chemicals were of analytical grade and were obtained from Methanor (Amsterdam, The Netherlands), Baker (Deventer, The Netherlands) and Rhône-Poulenc (Amstelveen, The Netherlands).

Drug assay

Unchanged eltoprazine in plasma and urine was measured by high-performance liquid chromatography. After addition of DU 121334 as internal standard, biologic samples and appropriate calibration standards were extracted on a preconditioned C-18 column (Baker 10 SPE no. 7020-3, Deventer, The Netherlands). After appropriate washing with water, the column was eluted with 2 ml methanol containing 2% of ammonia (25% w/w). The eluate was diluted with 2 ml of water and extracted with 4 ml of a mixture of dichloromethane and acetone (80:20 v/v). After centrifugation, the organic extract was separated into a vial of suitable volume with teflon-laminated disc, and evaporated to dryness at 35°C in a gentle stream of nitrogen.

The residue was redissolved in 200 μl of acetonitrile and 50 μl of a solution of the derivatization reagent dansyl chloride (a solution of 10 mg 5-dimethylamino-1-naphthalene-sulphonylchloride in 5 ml acetonitrile) was added. The reaction time was 10 min at room temperature. 500 μl of water and 50 μl potassium carbonate solution (60% w/v in water) were added and the reaction was continued for 5 min at 60°C .

After another extraction with 5 ml diethylether: *n*-pentane (80:20 v/v), the organic layer was

separated after rapid cooling in dry ice. After evaporation, an aliquot of the residue was dissolved in 100 μ l acetonitrile: H₂O (65:35 v/v) and was injected into the h.p.l.c. system (Waters Associates, model 6000 A, Milford, USA). This comprised a reversed phase (C-18) column and a fluorescence detector (Shimadzu, model RF535, Kyoto, Japan). The mobile phase was acetonitrile: H₂O (65:35 v/v) and its flow rate was 2 ml min⁻¹. The fluorescence detector was set at an excitation wavelength of 350 nm and an emission wavelength of 515 nm. The method was validated for linearity, and reproducibility (CV = $\pm$ 8% in plasma and urine) at concentrations up to 100 ng ml⁻¹ in plasma and 0–1 μ g ml⁻¹ in urine. The assay limit was 1 ng ml⁻¹ plasma and 0.05 μ g ml⁻¹ urine.

Pharmacokinetic analysis

Plasma and urine concentrations of eltoprazine were analysed by non-compartmental methods (Gibaldi & Perrier, 1982): $C_{\max}$ and $t_{\max}$ were taken directly from the concentration-time values. AUC was measured by the linear-logarithmic trapezoidal rule according to Chiou (1978) and Proost (1985), and extrapolated to infinity. MRT was calculated as AUMC/AUC with correction for the infusion time in the case of i.v. dosing. CL, $t_{1/2}$, F (AUC-ratio of 8 mg oral and 8 mg i.v.), $Ae(\infty)$ and V_{ss} were also calculated. Bioavailability was also calculated with correction for variable renal clearance according to Øie & Jung (1979). Renal clearance (CL_R) was calculated by dividing the urinary recovery of eltoprazine by the AUC.

Statistical analysis

Within-subject differences in pharmacokinetic parameters were tested using Student's paired t -test at the 5% level of significance. Only two of

the three treatments (8 mg i.v. and 8 mg p.o.) were given according to a balanced two period (study period 2 and 3) cross-over design, the 3 mg i.v. dose always being given in the first session. Thus treatment effects were confounded with session effects.

The data from sessions 2 and 3 were analysed by analysis of variance (ANOVA) specifying effects for subjects, treatments and sessions. The F -test for sessions did not attain significance at the 5% level for any of the parameters examined. Data from all sessions were analysed using ANOVA, specifying effects for subjects and treatments and assuming no session or carry-over effects. Contrasts tested (on a within subject basis) were i.v. 3 vs i.v. 8 mg and i.v. 8 vs p.o. 8 mg sessions. Statistical significance was set at 5%. The results are presented as arithmetic mean values $\pm$ s.d.

Results

After i.v. dosing, the disappearance of eltoprazine from plasma was biphasic, whereas after oral dosing decay appeared to be monophasic. Pharmacokinetic characteristics of the drug after i.v. and p.o. doses are summarised in Table 1. Mean terminal plasma half-lives were 7.0 and 9.3 h after i.v. doses of 3 and 8 mg, respectively, and were not significantly different. After oral administration, the mean terminal half-life was 9.8 h. Mean residence times (MRT) after i.v. administration of 3 mg (11.3 $\pm$ 4.9 h) and 8 mg (10.8 $\pm$ 2.1 h) were not significantly different. After oral dosing the mean MRT was 14.1 h. The mean elimination half-life ($t_{1/2}$ ur) estimated from urine data after i.v. dosing was higher than the corresponding value estimated from plasma data (11.1 h vs 7.0 h and 11.1 h vs 9.3 h after i.v. administration of 3 and 8 mg, respectively). No statistically significant differences were found in

Table 1 Pharmacokinetic parameters (mean $\pm$ s.d.) of eltoprazine

	Dose (mg)		
	3 i.v.	8 i.v.	8 p.o.
$t_{1/2}$ (h)	7.0 (3.1)	9.3 (4.4)	9.8 (3.9)
$t_{1/2}$ ur (h)	11.1 (5.1)	11.1 (5.4)	11.4 (6.8)
MRT (h)	11.3 (4.9)	10.8 (2.1)	14.1 (4.7)
$t_{\max}$ (h)	—	—	2.3 (1.1)
$C_{\max}$ (ng ml ⁻¹)	—	—	24 (6)
CL (ml kg ⁻¹ h ⁻¹)	487 (148)	471 (56)	—
CL _R (ml kg ⁻¹ h ⁻¹)	226 (124)	189 (38)	183 (81)
V_{ss} (l kg ⁻¹)	3.3 (0.7)	3.8 (0.5)	—
$Ae(\infty)$ (% of dose)	43 (12)	40 (3)	41 (19)
F (% of dose)	—	—	110 (32)

the $t_{1/2}$ ur values between the three treatments. Assuming that there were no session or carry-over effects, no statistically significant differences in pharmacokinetic parameters between treatments were observed. The mean values of CL and V_{ss} were 487 (3 mg i.v.) and 471 (8 mg i.v.) $\text{ml kg}^{-1} \text{h}^{-1}$ and 3.3 (3 mg i.v.) and 3.8 (8 mg i.v.) l kg^{-1} , respectively. No significant differences were found in CL_R values between the two i.v. doses. The mean contributions of renal clearance to total body clearance ranged from 35 to 44%. The mean cumulative urinary excretion ($A_e(\infty)$) ranged from 39 to 42% of the dose. Statistical analysis revealed no differences for the three treatments. The pH of the urine (range 5 to 8) did not appear to influence the elimination of eltoprazine.

The absolute bioavailability of eltoprazine was calculated to be $110 \pm 32\%$. For six subjects the bioavailability could be calculated in two ways, in the usual manner ($\text{AUC}_o/\text{AUC}_{iv}$) and according to Øie & Jung (1979). The results were $102 \pm 29\%$ and $95 \pm 16\%$, respectively. Values of dose-corrected mean AUC did not differ significantly ($P > 0.05$), suggesting dose proportionality of eltoprazine kinetics after i.v. administration of 3 and 8 mg to healthy male subjects.

No serious adverse reactions occurred. Somnolence was the only symptom which was rated as severe. In those instances there was a clear urge to sleep, but the subjects could be easily aroused after they had fallen asleep. The occurrence of somnolence depended on (i.v.) dose and route of administration. After oral dosing, three of 12 subjects experienced mild (1) or moderate (2) somnolence, while after i.v. dosing of 3 mg and 8 mg six of 12 (1 mild, 3 moderate, 2 severe) and seven of 12 subjects (2 moderate, 5 severe), respectively, experienced somnolence.

Other unwanted effects included asthenia (2 reports at 3 mg i.v.), heat sensation (1 report at 8 mg p.o.), headache (1 report at 8 mg p.o.), poor sleep at night (1 report at 3 mg i.v.), dizziness on rising (1 report at 3 mg i.v.), concentration problems (1 report at 8 mg p.o.), diarrhoea (1 report at 8 mg p.o.), hypotension (1 report at 8 mg i.v.), but they did not appear to be related to dose. None of these symptoms was judged to

be severe, required treatment or caused the subjects to withdraw from the study. In some instances the onset of complaints (headache or asthenia) was quite late, e.g. 48 or 72 h post-drug. The complaints of somnolence generally appeared within the first hour but had waned within 4-5 h after both oral and intravenous drug administration. No systematic effect on blood pressure, pulse rate or ECG was found. Laboratory values were within normal limits, except for a slight trend for an increase in blood urea over session days.

Discussion

This phase I study in 12 healthy male subjects has shown that the new serenic compound eltoprazine was well tolerated after single oral and intravenous doses up to 8 mg. Somnolence was the main complaint, which appeared to be dose related. Despite the fact that the intravenous doses were administered over a period of 1 h, which was estimated to be the earliest possible $t_{\max}$ of orally administered drug, the infused 3 mg dose induced more somnolence than the 8 mg oral dose. Since there was no placebo control it is difficult to be precise about the significance of this symptom. The values of systemic clearance ($471 \pm 56 \text{ ml kg}^{-1} \text{h}^{-1}$), mean residence time ($10.8 \pm 2.1 \text{ h}$) and steady-state volume of distribution ($3.8 \pm 0.5 \text{ l kg}^{-1}$) after dosing of 8 mg by constant infusion of 8 mg eltoprazine over 1 h indicated that eltoprazine is distributed extensively and cleared rather slowly from the body. Eltoprazine is poorly bound to plasma proteins ($15 \pm 5\%$ in the concentration range of 10 to 1000 ng ml^{-1}) (Duphar data on file). The renal clearance represented 40% of the total clearance. Its value ($\sim 200 \text{ ml kg}^{-1} \text{h}^{-1}$) suggests that eltoprazine is subject to tubular secretion. The complete oral bioavailability indicates that eltoprazine undergoes little first-pass metabolism. The elimination half-life of 10 h suggests that twice daily dosage may be appropriate.

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