PHARMACOKINETICS OF ELTOPRAZINE IN HEALTHY SUBJECTS

J. van Harten*, I.S. Mathlener and M. Raghoebar

Duphar B.V., Drug Disposition Department
P.O. Box 900, 1380 DA Weesp, The Netherlands

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*To whom correspondence should be addressed.

SUMMARY

The pharmacokinetics of eltoprazine in healthy male subjects was investigated after intravenous and oral dosing in one study, and after oral dosing of ^{14}C-eltoprazine in a second study. It was shown that the absorption of eltoprazine from the gastro-intestinal tract was complete, and that absolute bioavailability was 100%. The mean elimination half-life of eltoprazine in plasma was about 8 hours. Approximately 40% of the dose was excreted unchanged in urine.

I. INTRODUCTION

Eltoprazine hydrochloride (hereafter called eltoprazine) (Figure 1) is a phenylpiperazine derivative with 5-$HT_{1A/1B}$ agonistic properties which is currently developed for the treatment of pathological destructive behaviour associated with a variety of mental disorders.

As a part of the pharmacokinetic development programme, the pharmacokinetics of the drug after intravenous and oral administration (Study I) and after oral administration of ^{14}C-eltoprazine (Study II) to healthy male subjects was investigated.

II. MATERIALS AND METHODS

2.1 Medication

Intravenous infusion fluids (Study I) contained 3 mg or 8 mg eltoprazine in 500 ml saline. The fluid was sterile, and pyrogene-free. Oral doses of 8 mg (Study I) consisted of 3 capsules with 5, 2, and 1 mg eltoprazine, respectively, in a granulate.

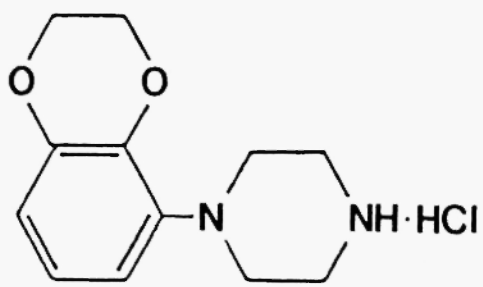

Fig. 1: Chemical structure of eltoprazine.

Oral doses of radiolabelled eltoprazine (Study II) consisted of capsules with 10 mg [phenyl-U-^{14}C]eltoprazine with a specific activity of 78.4 kBq per mg.

All medication was prepared by Duphar B.V.

2.2 **Subjects**

In the first study, 12 healthy male subjects, aged 20 to 37 years, and weighing between 56 and 88 kg, participated. In the second study, 8 healthy male subjects (age 21-31 years; weight 69-90 kg) participated. All participants gave written informed consent. Cardiovascular and other relevant diseases were excluded by physical examination, medical history, and appropriate laboratory tests. All subjects were non-smokers, and none was on any medication during the study or in the preceding two weeks. The protocols were approved by the investigators' independent Medical Ethics Committees.

2.3 **Study designs**

Study I was a three-way cross-over design in which eltoprazine was twice given as an intravenous infusion (3 mg and 8 mg in 1 hour), and once orally at a dose of 8 mg. For safety reasons, all subjects received the lowest dose (3 mg i.v.) first; the i.v. and oral doses of 8 mg were given in a randomized order. The interval between two study sessions was at least two weeks.

All experiments started at 8 a.m. after an overnight fast. An indwelling cannula with an obturator (Critikon®) for blood sampling up to 16 hours after dosing was inserted in an elbow vein. Infusions were given in the opposite arm.

Oral medication was taken with 250 ml of water, and 1 hour later another 250 ml was drunk. Blood samples (10 ml) for determination of plasma levels of eltoprazine were collected in heparinized tubes just before, and 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 hours after oral dosing or the start of the infusions. Urine for determination of unchanged eltoprazine was collected in separate fractions from 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 hours after drug administration. Plasma samples and aliquots of urine portions were stored at –20°C until analysis.

A light breakfast was supplied one hour before drug administration. Lunch was provided 4 hours, and dinner 12 hours after dosing. Caffeine or drinks containing alcohol were not allowed on study days.

In Study II, the participants received a single, 10-mg dose of ^{14}C-eltoprazine orally at 8 a.m. on the first study day, and they were institutionalised until not more than 0.1% of total radioactivity was excreted in urine in a 12-hour time interval. This was generally achieved within 5 days.

Blood samples for measurement of plasma radioactivity and plasma eltoprazine were taken just before, and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 10, 12, 16, 24, 36, 48, and 72 hours after dosing. Urine for determination of total radioactivity was collected in 16 separate fractions until 120 hours after dosing. Stools for determination of total radioactivity were collected until 120 hours after dosing, with a minimum of 5 stools.

Blood sampling technique, as well as food and fluid restrictions, were as in Study I. Food and fluid restrictions were lifted 12 hours after dosing.

2.4 **Drug Assays**

Plasma and urine concentrations of eltoprazine were determined with a specific HPLC-method, which was validated in terms of linearity and reproducibility (C.V. approximately 8%) up to 100 ng/ml in plasma and up to 1000 ng/ml in urine. The limit of quantification was 1 ng/ml (plasma) and 20-80 ng/ml (urine).

After addition of a structural analogue as internal standard, biological samples were extracted over a preconditioned C-18 extraction column (Baker 10 SPE no. 7020-3). 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 again 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 a mixture of 200 μl of acetonitrile and 50 μl of a solution of the derivatization reagent dansylchloride (solution of 10 mg 5N-dimethylaminonaphthalene-1-sulphochloride in 5 ml acetonitrile). The derivatization reaction time was ten minutes at room temperature; 500 μl of water and 50 μl potassium carbonate solution (60% in water) were added and the reaction continued for five minutes 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 μl acetonitrile: water (65:35 v/v) and injected on the HPLC-system.

This HPLC system consisted of a reverse-phase (C-18) column and a fluorescence detector. The mobile phase was acetonitrile: water (65:35). The mobile phase flow rate was 2 ml/min. The fluorescence detector was set at an excitation wavelength of 350 nm and an emission wavelength of 515 nm.

Radioactivity in plasma, urine, and faeces was measured by Liquid Scintillation Counting. Samples of 0.1 and 0.5 ml urine, made up with water to 1 ml, or 1.0 ml plasma were pipetted into counting vials and then mixed with 10 ml Emulsifier Scintillator 299 (Packard). The radioactivity was measured in a Philips liquid scintillation counter, type PW 4540 (Philips, Eindhoven, The Netherlands). The counting results were automatically corrected for quenching by an external standard technique.

Faeces were weighed and homogenized; weighed samples (100-400 mg) of the homogenates were burnt in a Packard Combustion Analyser (model 306). Carbon dioxide was trapped into a mixture of 8 ml fluid Carbosorb (Packard) and 12 ml Perma Fluor (Packard). The radioactivity of the resulting solutions was measured as described above. The results were corrected for incomplete recovery from combustion. The required correction factor was obtained by the combustion of specified amounts of [^{14}C]-labelled material dissolved in 0.1 ml water.

The urinary and faecal results were expressed as percentage of the dose, referring to the parent compound. The results of plasma level measurements are expressed in Bq per ml. The limit of quantification was 0.333 Bq/ml.

2.5 Methods of calculation

Pharmacokinetic parameters of unchanged eltoprazine were obtained either directly from the data points, or by model-independent statistical moment analysis /1/.

III. RESULTS

The mean (± S.D.) plasma concentration versus time curves of eltoprazine after intravenous (3 mg and 8 mg in 1 hour) and oral (8 mg) administration to 12 subjects are given in Figure 2.

After i.v. administration, a biphasic decline of plasma levels was observed. After oral administration of 8 mg, plasma levels of eltoprazine were comparable with plasma levels obtained after i.v. infusion of the same dose. The mean elimination rate after all three modes of administration was nearly identical.

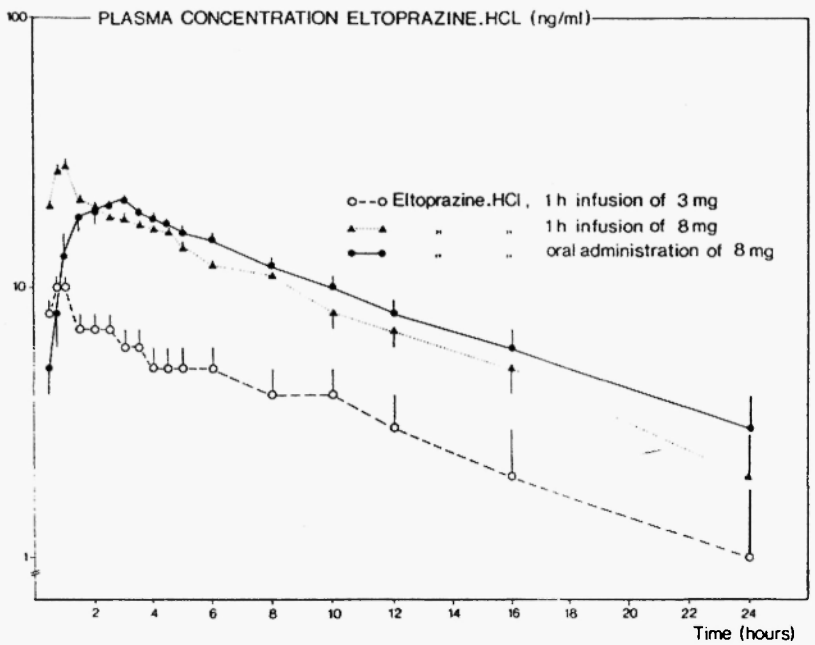

Fig. 2: Geometric mean (± S.D.) plasma concentrations of eltoprazine versus time in twelve healthy male subjects (data taken from /2/).

Mean (± S.D.) pharmacokinetic parameters after i.v. and oral eltoprazine are given in Table 1. Mean plasma clearance after i.v. and oral administration were almost the same, between 450 and 500 ml/h/kg. Volume of distribution was 3 to 4 l/kg, and mean residence time approximately 10 hours.

Approximately 40% of the dose was excreted as unchanged eltoprazine in urine, and renal clearance of the parent compound was calculated to be approximately 200 ml/min. Absolute bioavailability of oral eltoprazine was complete.

Mean plasma levels of unchanged eltoprazine, as well as mean plasma levels of total radioactivity and radioactivity originating from the parent compound are depicted in Figure 3. Plasma levels of total radioactivity were higher than levels due to unchanged eltoprazine. The contribution of eltoprazine to total plasma levels of radioactivity tended to decrease with time: during the first three hours after dosing, between 30 and 40% of plasma radioactivity could be attributed to metabolites of eltoprazine, whereas from 5

TABLE 1

Pharmacokinetic parameters of eltoprazine after oral and intravenous dosing in twelve healthy male subjects.
The number of evaluable data sets for each parameter is given in brackets.

		Mean ± S.D. after administration of eltoprazine					
		Intravenous					
		3 mg			8 mg		
$T_{1/2}$	(h)	7.0 ± 3.1	(n=9)		9.3 ± 4.4	(n=12)	
AUC	(ng.h/ml/kg)	96 ± 28	(n=9)		247 ± 30	(n=12)	
CL_s	(ml/h/kg)	487 ± 148	(n=9)		471 ± 56	(n=12)	
V_{ss}	(l/kg)	3.3 ± 0.7	(n=9)		3.8 ± 0.5	(n=12)	
MRT	(h)	10.1 ± 3.4	(n=9)		10.8 ± 2.1	(n=12)	
Ae	(% of dose)	43 ± 12	(n=11)		40 ± 3	(n=7)	
CL_R	(ml/h/kg)	226 ± 124	(n=9)		188 ± 42	(n=7)	
		Oral					
		8 mg					
$T_{1/2}$	(h)	9.8 ± 3.9		(n=12)			
T_{max}	(h)	2.3 ± 1.1		(n=12)			
C_{max}	(ng/ml)	24 ± 6		(n=12)			
AUC	(ng.h/ml/kg)	282 ± 80		(n=12)			
CL_{or}	(ml/h/kg)	457 ± 115		(n=12)			
MRT	(h)	14.1 ± 4.7		(n=12)			
F	(%)	110 ± 32		(n=12)			
Ae	(% of dose)	41 ± 19		(n=10)			
CL_R	(ml/h/kg)	183 ± 81		(n=10)			

hours after dosing and onwards, more than 50% of plasma radioactivity was due to metabolites.

Pharmacokinetic parameters of unchanged eltoprazine were not much different from parameters calculated in the i.v./p.o. study (Study I): mean (± S.D.) maximal plasma levels of eltoprazine after oral administration of 10 mg were 28 ± 7 ng/ml, and were reached after 3.1 ± 1.4 hours. Clearance was 448 ± 140 ml/h/kg, and mean residence time 9.5 ± 2.2 hours. The elimination half-life of eltoprazine was 7.0 ± 1.2 hours.

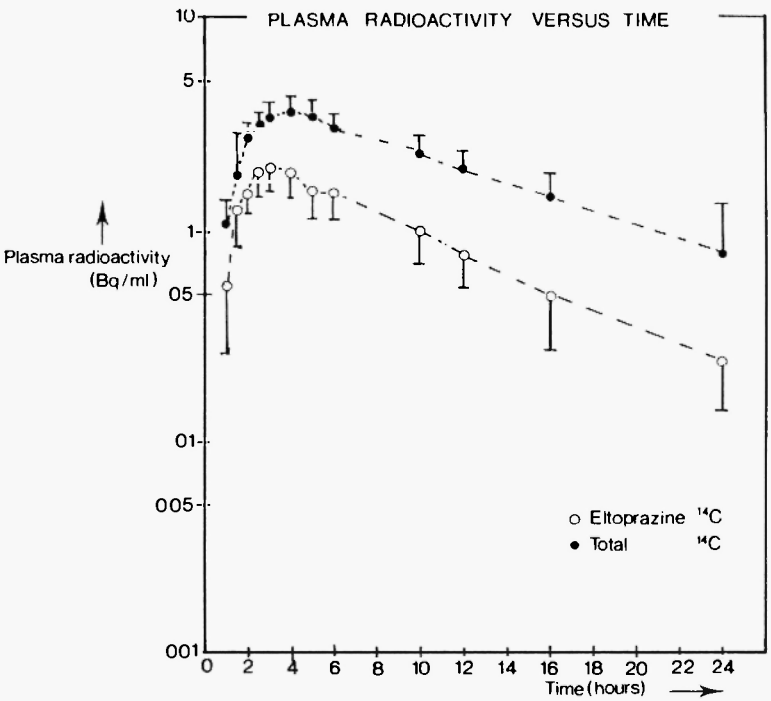

Fig. 3: Geometric mean (± S.D.) plasma levels of total plasma radioactivity (closed circles) and plasma radioactivity attributable to eltoprazine (open circles) versus time in eight healthy male subjects.

At the end of the five-day study period, a mean of 95.4% [range: 94.8-96.0%] of the radioactive dose was recovered. Of this 93.4% [range: 92.8-94.0%] appeared in urine; 1.9% [range: 1.6-2.2%] in faeces.

Urinary excretion of radioactivity within 24, 48, and 72 hours was 78.5 ± 5.4%, 91.1 ± 2.0%, and 92.9 ± 1.0% of the total dose, respectively.

IV. DISCUSSION

In the two studies presented here, the basic pharmacokinetics of eltoprazine after single-dose i.v. and oral administration to healthy

male subjects has been investigated. Both studies provided evidence that the absorption of eltoprazine from the gastro-intestinal tract is virtually complete. Orally administered radioactivity was almost completely excreted in urine within five days. Comparison of plasma concentration versus time profiles after i.v. and oral dosing revealed that not only absorption, but also absolute bioavailability, was complete.

The terminal half-life of eltoprazine was comparable in the two studies and in the i.v. and oral study sessions, which indicates that the terminal rate constant after oral administration really reflects the elimination process of the drug.

Volume of distribution was about 3 to 4 l/kg, indicating extensive distribution into aqueous and fatty tissue. Penetration into fatty tissue generally is not an instantaneous process, and in accordance with this is the observation that the decline of plasma concentrations of eltoprazine after i.v. administration was multiphasic (Figure 2). This profile was not observed after oral administration, but this apparently contradictory observation can be explained by the phenomenon of "vanishing exponentials", the phenomenon that a distribution phase is not recognized in an oral concentration-time-curve when the absorption rate constant is smaller than the distribution rate constant /3/.

Eltoprazine was excreted unchanged in urine to a large extent, and the calculated renal clearance of the drug exceeded the glomerular filtration rate (approx. 125 ml/min). It is probable that active renal tubular secretion plays a role in the elimination of the parent compound into urine, as also seems to be the case in dogs /4/.

From the present single-dose data, we conclude that no particular pharmacokinetic problems should be encountered in the future development of eltoprazine. From a pharmacokinetic viewpoint, a dosing frequency of two times daily seems appropriate during early clinical efficacy studies in patients.

V. ACKNOWLEDGEMENTS

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VI. REFERENCES

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