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Determinants of systemic availability of promethazine in rabbits

G. TAYLOR* AND J. B. HOUSTON

Department of Pharmacy, University of Manchester, Manchester M13 9PL, U.K.

Promethazine blood concentration-time curves have been determined in 7 rabbits following intravenous, oral and hepatic portal vein administration of promethazine. This phenothiazine has a large volume of distribution and a high metabolic clearance resulting in low blood concentrations particularly when the oral route is used. Analysis of the areas under the blood concentration-time curves indicates that hepatic first-pass metabolism is the major determinant of promethazine's low oral availability. Absorption from the gastrointestinal tract is essentially complete in most rabbits and the contribution of metabolism by the intestinal mucosa is minimal. The present findings are compared with the literature on other phenothiazines.

Promethazine (PMZ) is a widely used member of the phenothiazine group of drugs. It is employed in the treatment of allergic conditions and in a variety of other therapeutic areas for its anti-emetic, hypnotic and sedative properties.

In common with other phenothiazines, PMZ has an oral availability in man which is markedly less than unity (DiGregorio & Ruch 1980; Houston & Taylor 1981). It has become widely accepted that the incomplete oral availability of phenothiazines results from metabolism in the intestinal wall during absorption (Gibaldi & Perrier 1974; Lader 1976; Curry 1976; Cooper 1978; Davis et al 1978; Routledge & Shand 1979). The original studies (Curry et al 1971) which lead to this hypothesis were carried out in-vitro using everted gut sacs and subsequent in-vivo studies have provided conflicting results (Breyer & Winne 1977; Curry 1971; Dahl 1976; Dahl & Strandjord 1977; Houston & Taylor 1981; Schmalzing 1977). In addition it is known that phenothiazines are highly cleared from the body (Clark & Kaul 1976; Dahl 1976; Dahl & Strandjord 1977; Hansen et al 1976; Houston & Taylor 1981) via various metabolic routes. Hence it would be predicted that phenothiazines have a high hepatic extraction ratio and will undergo extensive hepatic first-pass metabolism following oral administration. Indeed a hepatic extraction ratio of 0·8 has been measured directly in-vivo for trifluoperazine in the rat (Schmalzing 1977).

In the present studies, we have administered PMZ to rabbits by intravenous, oral and hepatic portal vein dosing routes. Using this experimental methodology, we have been able to assess the relative contribution of first-pass metabolism by the hepatic and intestinal mucosal enzymes to the overall availability of PMZ. The rabbit was selected as an appropriate animal model for this study since this species has been documented to possess a high concentration of mucosal drug metabolizing enzymes (Chhabra et al 1974; Chhabra & Fouts 1976).

* Correspondence and present address: Welsh School of Pharmacy, U.W.I.S.T., Cathays Park, Cardiff CF1 3NU, U.K.

MATERIALS AND METHODS

Chemicals

[^{35}S]Promethazine hydrochloride (specific activity 8·4 mCi nmol^{-1}, radiochemical purity 99% by t.l.c.), promethazine hydrochloride and prochlorperazine mesylate were gifts from May and Baker (Dagenham, Essex). Reagents, of Analar grade where available, were obtained from BDH Chemicals (Poole, Dorset) with the exception of: isoamylalcohol (Aldrich Chemical Company, Gillingham, Dorset) and [^{35}S]dioctyl sulphide (Radio Chemical Centre, Amersham).

Animals and drug administration

A group of seven adult male New Zealand White/Half Lop cross-bred rabbits (2·1–3·4 kg) were used. Each rabbit received 0·1 μCi of [^{35}S]PMZ at a dose of 5 mg kg^{-1} by intravenous and oral routes using a balanced cross-over design with a one week interval between dosing. Four of the rabbits also received a similar dose of non-radioactive PMZ directly into the hepatic portal vein.

Before dosing, each rabbit was weighed and

injected subcutaneously with atropine sulphate (0·1 mg kg^{-1}). After 20 min, sedation was effected by intramuscular injection of Hypnorm 0·5 ml kg^{-1} (Janssen Pharmaceuticals Limited). Sedation was maintained by subsequent doses (0·25 ml kg^{-1}) of Hypnorm.

Intravenous doses, consisting of 4 ml PMZ solution made isotonic with NaCl, were infused over 5 min into a similar catheter to that used for blood sampling located in the alternate ear. Portal vein doses consisted of a similar solution infused over a 5 min period through a 25-G Butterfly catheter (Abbot Laboratories Limited) inserted directly into the hepatic portal vein, and held in place using cyanoacrylate adhesive. Immediately after the dose had been given this catheter was flushed with 2 ml of 0·9% NaCl (saline). Oral doses of 1 ml were given via an oesophageal tube. Samples of each dosing solution were assayed for PMZ and radioactivity.

For the collection of blood samples, an ear was shaved and the marginal ear vein catheterized (Abbocath T 22-G, Abbot Laboratories Limited). A predose blood sample (1 ml) was collected and after this and each subsequent sample the catheter flushed with 0·5 ml of heparinized saline (125 i.u. ml^{-1}). Other blood samples were collected 15, 30, 45, 60, 90, 120, 180, 240, 360, 480 and 600 min after dosing. Urine samples were collected for 54 h after dosing using a square Perspex collecting funnel suspended beneath the holding cage. Separation of faeces was achieved by using a square mesh in the apex of the funnel.

Analysis of blood and urine samples

Rabbit blood and urine samples were assayed for PMZ by h.p.l.c. (Taylor & Houston 1982) with the following modifications: the eluent consisted of methanol containing 18% of 0·01 M Sorensen's pH 7·8 phosphate buffer. Eluent flow was maintained at 1·6 ml min^{-1} through a stainless steel column (200 mm × 4·8 mm i.d.) packed with Spherisorb 5-ODS (Phase Separations, Queensferry, Clwyd). Blood and urine samples (1·0 ml) were spiked with prochlorperazine mesylate (1·0 ml, 3·2 μg ml^{-1}) as internal standard, made alkaline with 0·5 ml of 1 M NaOH, and mixed with 5·0 ml of n-heptane containing 10% dichloromethane and 1·5% isoamylalcohol for 15 min using an inversion mixer. Following centrifugation (3000 *g*, 10 min) 4·8 ml of the organic phase was transferred to a nipple tube, and the extract mixed with 50 μl of 0·1 M HCl. The minimum detectable amount of PMZ using this procedure was 20 ng ml^{-1} of sample.

Urine samples were assayed for radioactivity using a Packard Tricarb (Model 2045) scintillation counter. Calibration was effected by the use of an internal standard quench correction procedure, [^{35}S]dioctyl sulphide being used as the internal standard. The scintillation cocktail consisted of Triton X-100: toluene (1:2) containing 4 g $litre^{-1}$ PPO and 0·1 g $litre^{-1}$ POPOP. 1·0 ml urine samples were added to 10 ml of the scintillation fluid.

Determination of pharmacokinetic parameters

Areas under the blood concentration-time curves and under the first moment curves from zero to infinity were calculated using linear trapezoidal summation with appropriate extrapolations (Gibaldi & Perrier 1975; Benet & Galeazzi 1979) using half-lives regressed from terminal blood concentration data. Blood clearance and steady-state volumes of distribution were calculated from the areas obtained following intravenous administration of PMZ.

The availability of PMZ after oral and hepatic portal vein administration was calculated from the ratio of the respective area under the curve to that area obtained for the intravenous route in the same rabbit. The extent of absorption from the gastrointestinal tract was assessed by the ratio of radioactivity recovered in the urine after oral and intravenous dosing of [^{35}S]PMZ.

RESULTS AND DISCUSSION

A typical blood concentration-time profile for PMZ following intravenous dosing is shown in Fig. 1. A

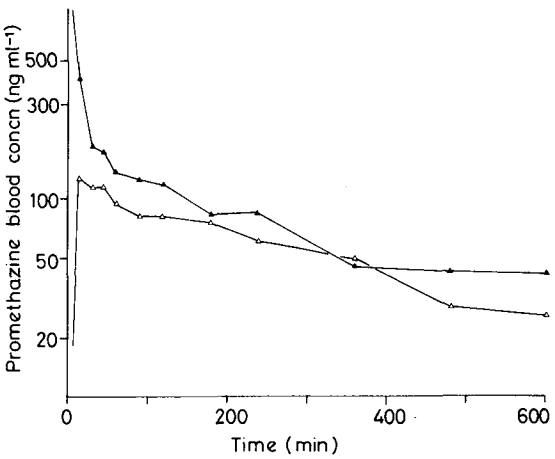

FIG. 1. Blood concentration-time profile for promethazine following intravenous (▲) and oral (△) administration of promethazine to a rabbit.

rapid 'distribution phase' lasting between 60 and 120 min was followed by a much slower 'elimination phase' which lasted for the remainder of the sampling period. The estimates of PMZ half-life in the 'elimination phase', $\beta t_{1/2}$, are presented in Table 1, together with other pharmacokinetic parameters. The mean value for $\beta t_{1/2}$ was 309 min, the sampling period of 600 min representing about two terminal half-lives.

Table 1. A summary of the disposition parameters of promethazine in the rabbit.

Blood clearance (ml min^{-1} kg^{-1})	56·3 ± 12·3
Steady-state volume of distribution (litres kg^{-1})	20·2 ± 5·5
β-half-life (min)	309 ± 96
Oral availability (% of dose)	49 ± 20
Oral absorption (% of dose)	81 ± 15

Mean ± s.d. for 7 rabbits.

The total body clearance for PMZ showed inter-animal variation between 33 and 67 ml min^{-1} kg^{-1}, the clearance is almost wholly metabolic as less than 1% of the dose is excreted as unchanged PMZ (Table 3). The steady-state volume of distribution is large (17·1–33·7 litre kg^{-1}), being similar to that calculated for man (Houston & Taylor 1981) and is indicative of extensive tissue binding of PMZ.

After oral dosing, peak concentrations of PMZ were reached very rapidly as typified in Fig. 1. Maximum concentrations, measured 15 min after dosing, varied between 90 and 290 ng ml^{-1} and were lower than the corresponding concentrations after intravenous dosing. A 'distribution phase' was observed lasting between 60 and 180 min, this was followed by an 'elimination phase'. The half-life of PMZ in this latter phase was within 10% of the corresponding value measured after intravenous dosing.

The areas under the PMZ blood concentration-time curve between zero and infinity (AUC) after oral and intravenous administration of PMZ are presented in Table 2. The AUC after oral dosing shows a statistically significant reduction ($P < 0·01$) to approximately half of that after intravenous dosing. The fraction of the oral dose reaching the systemic circulation as unchanged drug (F), is influenced by that fraction of the dose absorbed (f_A), and that fraction of the dose escaping first-pass metabolism in the gut wall (f_G) and liver (f_H):

$$F = f_A \cdot f_G \cdot f_H$$

Blood concentrations of PMZ after hepatic protal vein dosing were lower than those following intravenous dosing. The concentrations declined in a biphasic manner, a rapid distribution phase lasting between 45 and 90 min. The half-life of PMZ in the second phase was the same as that measured after intravenous and oral dosing. A comparison of AUC for the intraportal and intravenous routes (Table 2) allows direct calculation of the fraction of dose escaping hepatic first pass metabolism (f_H). A mean value of 0·60 was obtained.

Table 2. Influence of route of administration on promethazine area under the blood concentration-time curve.

	Route of administration		
Rabbit	Intravenous	Oral	Hepatic portal
A	80·8	54·6	—
B	87·5	60·2	59·8
C	105·1	40·4	45·5
D	74·7	31·6	—
E	77·9	19·2	32·1
F	78·1	51·9	71·9
G	153·4	56·9	—
Mean ± s.d.	93·9 ± 28·1	45·0 ± 15·2	52 ± 17·3

The urinary excretion of PMZ, norpromethazine (Nor_1PMZ), promethazine sulphoxide (PMZ SO) and total radioactivity, after oral and intravenous dosing is shown in Table 3. Although urinary recovery of radioactivity is substantial, the average amounts excreted as PMZ, Nor_1PMZ and PMZ SO totals less than 1% of the dose for both routes. The ratio of radioactivity recovered after oral and intravenous PMZ administration allows calculations of the extent of absorption from the gastrointestinal tract. From the calculated values of F and f_A, the fraction of the absorbed oral dose escaping first-pass metabolism ($f_G \cdot f_H$) may be estimated. In Fig. 2A the relationship between F and $f_G \cdot f_H$ for the 7 rabbits studied is shown. The data points are situated around the mean f_A of 0·81 and they show that the major source of incomplete availability is first-pass

Table 3. Influence of route of administration of promethazine on the urinary excretion of promethazine (PMZ), monodesmethyl-promethazine (Nor_1 PMZ), promethazine sulphoxide (PMZ SO) and total radioactivity in the rabbit.

	Route of administration	
	Intravenous	Oral
PMZ	0·20 ± 0·34	0·20 ± 0·23
Nor_1 PMZ	0·07 ± 0·06	0·17 ± 0·33
PMZ SO	0·66 ± 0·22	0·39 ± 0·14
^{35}S	75·9 ± 13·7	60·4 ± 12·5

Mean ± s.d. for 7 rabbits (% of dose).

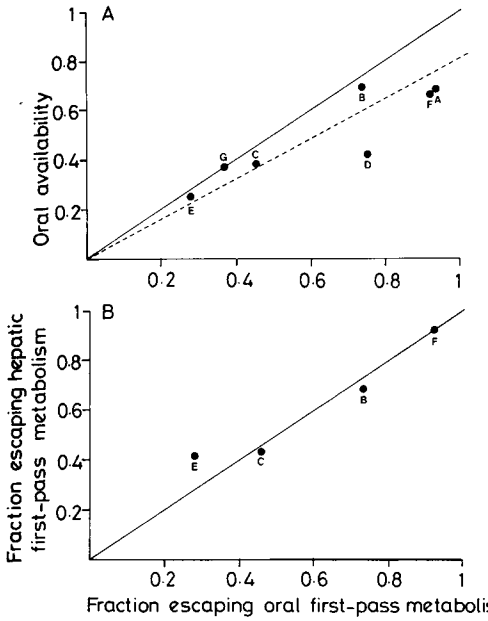

FIG. 2. A. Relationship between oral availability of promethazine (F) and fraction escaping oral first-pass metabolism (f_H . f_G) in 7 rabbits. Solid line is the slope of unity and dashed line the slope of 0·81 (the average f_A).

B. Relationship between fraction escaping hepatic first-pass metabolism (from hepatic portal vein administration) and fraction escaping oral first-pass metabolism (f_H . f_G). The line of identity is shown.

metabolism. Only in 3 rabbits (A, F and D) is absorption less than 80% of the administered dose.

In Fig. 2B the oral first-pass metabolism parameter (f_H . f_G) is plotted against f_H from the hepatic portal vein administration study in the same rabbit. In 3 of 4 rabbits there is good correspondence between these 2 parameters indicating that f_G is close to unity. The intraportal studies confirm the large inter-animal variation in first-pass metabolism observed after oral dosing.

Gut wall first-pass metabolism has been proposed as the major determinant for the low availability of phenothiazine drugs. In-vitro studies have shown the capability of the intestinal mucosa to metabolize these compounds (Curry et al 1971; Minder et al 1971) however in-vivo studies involving hepatic portal vein sampling (Breyer & Winne 1977; Schmalzing 1977) indicate minimal metabolism during absorption. Our findings are consistent with the latter in-vivo investigations.

It has been reported by Dahl & coworkers (Dahl 1976; Dahl & Strandjord 1977) that sulphoxide metabolites of methotrimeprazine and chlorpromazine are detectable in the plasma after oral but not intramuscular administration of the parent phenothiazine to man. These observations have been interpreted as evidence for *S*-oxidation by the intestinal mucosa. We (Houston & Taylor 1981) were unable to confirm these observations with promethazine in man and have proposed that the inability to detect sulphoxides after intramuscular administration is due to the kinetics of metabolite accrual.

In conclusion, the rabbit studies with promethazine have confirmed our previous investigations in man and indicate minimal intestinal wall metabolism during absorption. The major determinant of the low availability of PMZ is hepatic metabolism. This is in agreement with studies using perazine (Breyer & Winne 1977) and trifluoperazine (Schmalzing 1977) but at variance with the conclusions of other investigators (Curry 1976; Dahl 1976; Dahl & Strandjord 1977) that metabolism by the intestinal mucosa is a major determinant of the availability of phenothiazines.

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