J. Pharm. Pharmacol. 1985, 37: 243–247
Received July 15, 1984

Changes in the disposition of promethazine during multiple dosing in the rabbit

G. TAYLOR* AND J. B. HOUSTON

Department of Pharmacy, University of Manchester, M13 9PL, UK

During a multiple dosing regimen, the area under the promethazine blood concentration-time profile progressively decreased indicating auto-induction of metabolism. The increase in promethazine clearance (mean 35%) was not reflected in changes in either elimination half-life or minimum blood concentrations during the dosing interval. This was attributed to a deep compartment in the disposition of promethazine in the rabbit. The changes in promethazine clearance were accompanied by proportionally larger changes in the clearance of the monodesmethylpromethazine metabolite. The effect of promethazine pretreatment on the clearance of antipyrine was also studied and was found to be significantly increased by a mean of 17% following pretreatment with promethazine. However, the changes in the clearance of antipyrine did not highly correlate with those of promethazine and monodesmethylpromethazine. This may indicate that promethazine induces metabolic systems in the rabbit for which antipyrine is not a good substrate.

Promethazine (PMZ), a member of the phenothiazine group of drugs, has attracted widespread use over a number of years. PMZ's major use is for its antihistaminic and sedative properties when it is often dosed repeatedly for a number of weeks.

In man, multiple dosing of another phenothiazine drug, chlorpromazine, has been reported to result in decreased steady-state concentrations (Loga et al 1975; Sakalis et al 1972) and a shortening of antipyrine half-life (Kolakowska & Franklin 1975; Loga et al 1975). In-vitro studies indicate that microsomal enzyme induction occurs after chlorpromazine multiple dosing in rats (Breyer 1972; Aurori & Vesell 1974) indicating that auto-induction of metabolism is the mechanism responsible for the decrease in steady-state concentrations in man. In-vitro studies with PMZ have also demonstrated that in the rat pretreatment with this phenothiazine produces an induction of drug metabolism enzyme systems (Fernandez & Castro 1977). Recently (Taylor et al 1985) we have demonstrated that the PMZ induction profile in the rat has characteristics of both phenobarbitone- and polycyclic aromatic hydrocarbon-types of induction. PMZ pretreatment in the rat has also been shown to result in an increase of in-vivo drug metabolizing capacity, assessed using an aminopyrine breath test (Houston et al 1981).

In the present study the consequences of repeated dosing with PMZ on its own disposition and on that of antipyrine in the rabbit have been investigated.

* Correspondence and present address: Welsh School of Pharmacy, UWIST, PO Box 13, Cardiff, CF1 3XF, UK.

MATERIALS AND METHODS

Chemicals

Promethazine hydrochloride was kindly supplied by May and Baker (Dagenham, Essex). Monodesmethylpromethazine (Nor_1PMZ) and imipramine were gifts from Kabi Pharmaceuticals (Stockholm, Sweden) and Berk Pharmaceuticals (Guildford, Surrey). Promethazine sulphoxide (PMZSO) was prepared in the laboratory as described previously (Taylor & Houston 1982). Reagents, of Analar grade when available, were obtained from BDH Chemicals (Poole, Dorset) with the exception of isoamylalcohol which was obtained from Aldrich Chemical Company (Gillingham, Dorset).

Animals and drug administration

Each of a group of six adult male New Zealand White/Half Lop cross-bred rabbits (2·7–3·3 kg) received 14 doses of PMZ (10 mg kg^{-1}), given by intramuscular injection, at intervals of 24 h. During the 2nd, 8th and 14th days of the dosing regimen, serial blood samples (1·0 ml) were taken before and at 15, 30, 45, 60, 90, 120, 180, 240, 360, 480 and 600 min after dosing. The samples were assayed for PMZ, Nor_1PMZ and PMZSO, as described later.

In four rabbits antipyrine (10 mg kg^{-1}) was administered by intravenous injection, three days before and one day following, the PMZ dosing regimen. Blood samples (0·5 ml) were collected before, and at 15, 45, 75, 105, 135, 165 and 195 min after dosing. All blood samples were collected by the following method. Sixteen hours before the predose sample, each rabbit was weighed and given atropine

sulphate (0·1 mg kg^{-1}) subcutaneously. Twenty minutes later the animal was sedated with Hypnorm (Janssen Pharmaceuticals) 0·3 ml kg^{-1} by intramuscular injection. An ear was then shaved and the marginal ear vein catheterized (Abbocath T 22-G, Abbott Laboratories). Following the collection of each blood sample the catheter was flushed with 0·5 ml of heparinized saline (125 units ml^{-1}).

Analysis of blood and plasma samples

Rabbit blood samples were assayed for PMZ, Nor$_1$PMZ and PMZSO using a modification of an HPLC assay previously described (Taylor & Houston 1982). Briefly, each blood sample (1·0 ml) was spiked with imipramine hydrochloride (1·0 ml, 1·35 μg ml^{-1}) as internal standard, made alkaline with 0·5 ml of 1·0 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 using a vortex mixer. 10–20 μl aliquots of the aqueous phase were injected onto the HPLC column (100 mm × 4·8 mm i.d. containing Hypersil 5-SAS, Shandon Southerns, Runcorn, Cheshire). The minimum detectable concentrations of PMZ, Nor$_1$PMZ and PMZSO using this procedure were 5, 5 and 10 ng ml^{-1} respectively.

Rabbit plasma samples were assayed for antipyrine using the following method. To each 0·1 ml of plasma was added acetonitrile containing 2 μg ml^{-1} of phenacetin as internal standard and mixed using a vortex mixer for 15 s. The samples were then centrifuged at 3000*g* for 15 min and 15–20 μl aliquots of the supernatant injected onto the HPLC column. The eluent consisted of 0·4 M ammonium acetate containing 15% acetonitrile. Eluent flow was maintained at 2·0 ml min^{-1} through a stainless steel column (200 mm × 4·8 mm i.d.) packed with Spherisorb 5-ODS (Phase Separations, Queensferry, Clwyd). Detection was effected by u.v. monitoring at 254 nm. The interday coefficient of variation was found to be 2·5% at 2 μg ml^{-1} antipyrine.

Areas under the blood concentration-time profiles (AUCs) for PMZ and Nor$_1$PMZ were calculated using linear trapezoidal summation. The AUCs associated with a dosing interval were calculated using the predose concentrations at the end of the dosing interval. The clearance of PMZ was determined from the ratio of PMZ dose/AUC of PMZ during a dosing interval. The ratio of PMZ dose/AUC of Nor$_1$PMZ during a dosing interval, we have described as the 'relative clearance' of Nor$_1$PMZ (Houston 1981). This parameter is equivalent to the total body clearance of Nor$_1$PMZ/the fraction of the PMZ dose eliminated via this metabolite. Other pharmacokinetic parameters were calculated using standard methodology.

RESULTS

The mean blood concentration-time profile for PMZ during the second day of the dosing regimen, together with the corresponding blood concentration-time profile for Nor$_1$PMZ are presented in Figures 1 and 2. In each rabbit peak PMZ blood concentrations were attained within 30 min after dosing, subsequently, the concentrations declined in a biphasic manner. The elimination half-life of PMZ during the second phase (120–600 min) had a mean value of 228 min (Table 1). The extrapolated 24 h blood concentrations of PMZ based on this half-life were lower than those measured at the end of the first dosing interval in each of the rabbits studied. This may be indicative of a terminal half-life much longer than that observed during the period of 120 to 600 min after dosing.

The total body clearance of PMZ on the second day of the dosing regimen for the group is shown in Table 1. The mean value of 59·9 ml min^{-1} kg^{-1} was not significantly different (*t*-test) from that of 56·3 ml min^{-1} kg^{-1} measured following intravenous dosing (Taylor & Houston 1983). This indicates com-

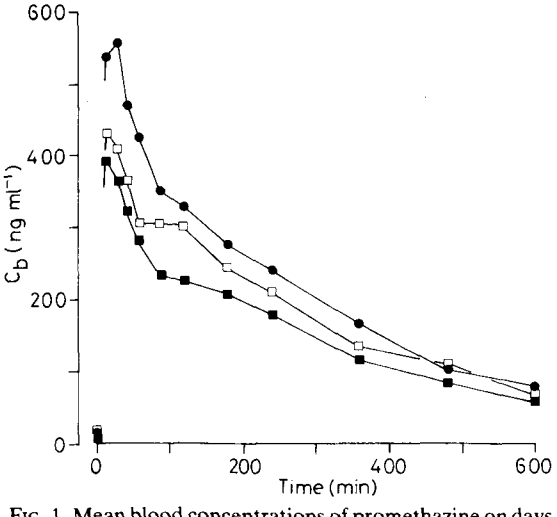

FIG. 1. Mean blood concentrations of promethazine on days 2 (●——●), 8 (□——□) and 14 (■——■) of a multiple dosing regimen.

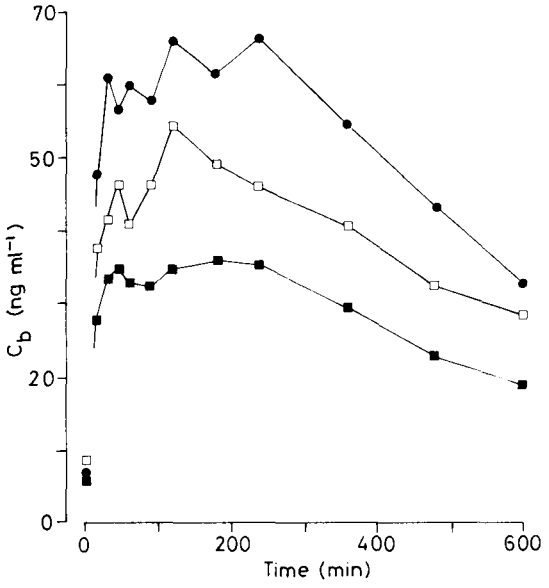

FIG. 2. Mean blood concentrations of monodesmethylpromethazine on days 2 (●——●), 8 (□——□) and 14 (■——■) of a promethazine multiple dosing regimen.

plete availability of PMZ following intramuscular dosing.

The blood concentrations of Nor_1PMZ showed an initial rapid increase to between 60 and 90% of peak concentrations within 30 min after dosing. Subsequently, a much slower increase to the maximum concentration (group range: 50 to 120 ng ml^{-1}, occurring between 90 and 240 min after dosing) was observed. Concentrations between 240 and 600 min after dosing declined monoexponentially. The elimination half-life of Nor_1PMZ during this phase had a mean value of 352 min (Table 1) and was longer than that of PMZ in each of the rabbits studied.

Concentrations of PMZSO were below 20 ng ml^{-1} throughout the sampling period and in most samples were below the assay limit of 10 ng ml^{-1}.

Blood concentrations of PMZ were lower on the 8th and 14th than on the 2nd day of the dosing regimen, as summarized in Fig. 1. Areas under the blood concentration-time profiles during the 8th and 14th dosing intervals were lower than that during the 2nd dosing interval. This change was reflected by a statistically significant ($P < 0.05$) increase in total body clearance of PMZ, from a mean value of 59·9 ml min^{-1} kg^{-1} during the 2nd day to 78·0 ml min^{-1} kg^{-1} on the 14th day. An intermediate clearance of 65 ml min^{-1} kg^{-1} was measured on the 8th day. There was no change in the elimination half-lives of PMZ and Nor_1 PMZ during 14 days of pretreatment. Neither were there any significant changes in predose PMZ and Nor_1 PMZ blood concentrations during the dosage regimen (Table 1).

Blood concentrations, and consequently areas associated with the blood concentration-time profile of Nor_1PMZ, were also lower during the 8th and 14th than on the 2nd day, as summarized in Fig. 2. The total body clearance of Nor_1PMZ cannot be calculated from these data alone, but a 'relative clearance' term equivalent to the total body clearance/the fraction of the PMZ dose eliminated via this metabolite is reported in Table 1. A statistically significant ($P < 0.05$) increase in the relative clearance of Nor_1PMZ was noted from 230 ml min^{-1} kg^{-1} during the 2nd day to 380 ml min^{-1} kg^{-1} during the 14th day of the dosing regimen. The relative clearance on the 8th day was 270 ml min^{-1} kg^{-1}.

Following intravenous dosing with antipyrine, plasma concentrations declined monoexponentially with no apparent distribution phase. The total body clearance of antipyrine showed a statistically significant ($P < 0.01$) increase from 8·44 (s.d. 1·16) ml min^{-1} kg^{-1} before, to 9·89 (s.d. 1·38) ml min^{-1} kg^{-1} after multiple dosing with PMZ. A significant increase ($P < 0.05$) was also noted in the apparent volume of distribution from 0·898 (s.d. 0·025) litre kg^{-1} before, to 1·002 (s.d. 0·026) litre kg^{-1} after PMZ pretreatment. The half-life was decreased by a small but insignificant amount from 75 (s.d. 11) min before to 71 (s.d. 9) min after the PMZ dosing regimen.

Table 1. Changes in the pharmacokinetics of promethazine and monodesmethylpromethazine during multiple dosing with promethazine.[a]

	Day of treatment		
	2	8	14
Promethazine			
Clearance (ml min^{-1} kg^{-1})	59·9 ± 15·2	65·0 ± 7·8	78·0 ± 16·4*
Half-life (min)	228 ± 28	249 ± 63	260 ± 51
Minimum blood concentration (ng ml^{-1})	19·5 ± 7·1	22·1 ± 6·4	19·8 ± 10·6
Monodesmethylpromethazine			
Relative clearance[b] (ml min^{-1} kg^{-1})	229·9 ± 77·6	269·6 ± 69·8	379·9 ± 109·6*
Half-life (min)	352 ± 50	450 ± 81	418 ± 151
Minimum blood concentration (ng ml^{-1})	7·2 ± 2·6	8·9 ± 4·1	6·3 ± 2·6

[a] Mean ± s.d. for six rabbits.
* Significantly different ($P < 0.01$) from day 2 and day 8 values using Student–Newman–Keuls test.
[b] See text for definition.

DISCUSSION

The decreases in AUC indicate increases in the clearance of PMZ during multiple dosing in the rabbit. Since the clearance of PMZ is almost wholly metabolic (Taylor & Houston 1983) these observations are congruent with the effects of PMZ on microsomal enzyme systems (Fernandez & Castro 1977; Taylor et al 1985) and indicate that PMZ is capable of auto-induction. The changes in clearance of PMZ were not reflected by changes in elimination half-life. This is not anomalous since PMZ is extensively distributed in the rabbit (Taylor & Houston 1983) and half-life has been shown to be a very poor index of hepatic elimination for such a drug (Perrier & Gibaldi 1974). Decreases in the minimum blood concentrations of PMZ would be expected to result from the observed increases in clearance. However, the observation that predicted predose concentrations on day 3 of the dosing regimen were consistently lower than those measured on day 2, may indicate a slow distribution of PMZ into a deep compartment. Such a phenomenon has been suggested to occur with chlorpromazine (Curry 1976). A slow distribution of PMZ would lead to increases in predose concentrations during a multiple dosing regimen. Thus the effects of multiple dosing would tend to negate any decrease in predose blood concentration resulting from metabolic induction. Increases in PMZ concentrations at other times during the dosing interval would be negligible since the fraction of the PMZ dose distributing into this deep compartment is small (less than 3% of the total area under the PMZ blood concentration-time profile was associated with the period between 10 and 24 h after dosing).

Since the relative clearance of Nor_1PMZ increased during multiple dosing with PMZ, we may conclude that either the clearance of the metabolite was increased or the fraction of the PMZ dose eliminated via this metabolite was decreased. Estimation of the fraction of PMZ metabolized by this route was not possible, since, from published data on other phenothiazines, we would predict Nor_1PMZ to be further metabolized. The PMZ dose fraction excreted in the urine as Nor_1PMZ is less than 1% in the rabbit (unpublished data), this is in accord with the concept of Nor_1PMZ as an intermediary metabolite. The change in relative clearance of Nor_1PMZ was proportionally greater than that of PMZ in each of the rabbits studied (Fig. 2). It has been suggested (Levy et al 1983) that an increase in metabolite clearance is necessary for such an effect to occur. Furthermore, a change (either increase or decrease) in the fraction of PMZ eliminated by demethylation may also have occurred, but this could not be ascertained without administration of the metabolite. The half-life associated with the decline in Nor_1PMZ concentrations was greater than the elimination half-life of PMZ and thus represents the true elimination half-life of the metabolite. A decrease in metabolite half-life was not observed during the study, however, as with the parent drug, changes in Nor_1PMZ half-life would only reflect clearance changes if the metabolite was not extensively distributed throughout the body. Nor_1PMZ has similar physicochemical properties to PMZ and hence would be expected to show an extensive distribution. The lack of change in predose Nor_1PMZ concentrations may also be attributed to extensive distribution as discussed previously for PMZ.

Antipyrine is commonly employed as an index of drug metabolizing capacity as it has a low total body clearance which is mostly metabolic. Furthermore, it is minimally bound to proteins in plasma and tissues and, as such, changes in its half-life should be a good indicator of changes in its clearance. In this study a significant increase was noted in its clearance, however, the corresponding decrease in half-life was not significant. The reason for this anomaly is due to the observed change in the apparent volume of distribution of antipyrine, which has been reported by others following metabolic induction in the rabbit (McManus & Ilett 1979). The half-life of antipyrine is a direct consequence of its clearance and volume of distribution and thus the significant increase in the latter opposed any changes in the half-life which would have been evident had the volume not changed.

The change in antipyrine clearance was not as great as that of PMZ (mean changes 17 and 35% respectively). This is contrary to what would be predicted, as the clearance of antipyrine is much lower and would be expected to change more than that of PMZ (Perrier & Gibaldi 1974). A comparison of the clearance of antipyrine before, and of PMZ at the beginning of, the multiple dosing regimen, in each of the rabbits, revealed a significant negative correlation ($r = 0{\cdot}96$, $P < 0{\cdot}05$). Furthermore, whilst the changes in PMZ and Nor_1PMZ clearances after multiple dosing were highly correlated ($r = 0{\cdot}97$, $P < 0{\cdot}05$), there was no relation between changes in the clearance of antipyrine and that of PMZ ($r = 0{\cdot}47$). This may indicate that PMZ induces metabolic systems in the rabbit for which antipyrine is not a good substrate.

In conclusion, AUCs for PMZ and Nor_1PMZ were

both decreased following multiple doses of PMZ. The results indicate that PMZ induces its own metabolism and that of its monodemethylated metabolite. PMZ was also noted to increase the clearance of antipyrine in the rabbit. These results demonstrate that the previously reported effects of PMZ on microsomal enzyme systems are manifest as in-vivo changes in drug clearance.

Acknowledgement

G. T. was a grateful recipient of an SERC CASE award with May and Baker Limited.

REFERENCES

Aurori, K. C., Vesell, E. S. (1974) Drug Metab. Dispos. 2: 566–572

Breyer, U. (1972) Naunyn-Schmiedeberg's Arch. Pharmacol. 272: 277–288

Curry, S. H., (1976) Br. J. Clin. Pharmacol. 3: Supplement, 20–28

Fernandez, G., Castro, J. A. (1977) Drug Metab. Dispos. 5: 91

Houston, J. B. (1981) Pharmacol. Ther. 15: 521–552

Houston, J. B., Lockwood, G. F., Taylor, G. (1981) Drug Metab. Dispos. 9: 449–455

Kolakowska, T., Franklin, M. (1975) Br. J. Clin. Pharmacol. 2: 25—28

Levy, R. H., Lane, E. A., Guyot, M., Brachet-Liermain, A., Cenraud, B., Loiseau, P. (1983) Drug Metab. Dispos. 11: 286–292

Loga, S., Curry, S. H., Lader, M. H. (1975) Br. J. Clin. Pharmacol. 2: 197–208

McManus, M. E., Ilett, K. F. (1979) Xenobiotica 9: 107–118

Perrier, D., Gibaldi, M., (1974) J. Pharmacol. Exp. Ther. 191: 17–24

Sakalis, G., Curry, S. H., Mould, G. P., Lader, M. H. (1972) Clin. Pharmacol. Ther. 13: 931–946

Taylor, G., Houston, J. B. (1982) J. Chromatogr. 230: 194–198

Taylor, G., Houston, J. B. (1983) J. Pharm. Pharmacol. 35: 284–288

Taylor, G., Elcombe, C. R., Houston, J. B. (1985) Xenobiotica 15: in the press