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Relative bioavailability of trimeprazine tablets investigated in man using HPLC with electrochemical detection*

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The stability, partition coefficient, plasma protein binding, red blood cell distribution, and whole blood concentrations of trimeprazine were investigated. Trimeprazine solution was stable for 6 months at −20 °C and 3·5 months at 40 °C. In whole blood trimeprazine was stable for 5 weeks at −20 °C, 24 h at 4 °C, 4 h at 25 °C and 1 h at 37 °C. The apparent hexane–water partition coefficient varied from 1·50 (at pH 4·83) to over 100 (at pH 10·54). The fraction bound to plasma protein exceeded 0·9 as estimated by equilibrium dialysis with correction for volume shift. The mean plasma/red blood cell concentration ratio was 1·17 and the mean red blood cell/plasma distribution coefficient was 8·65. Six healthy adult males received single 5 mg doses of trimeprazine in a syrup (5 mg in 10 ml) and tablets with at least two weeks between doses. Blood was collected for 48 h. The mean (±s.e.m.) times for peak blood concentrations were 3·5 ± 0·22 h for the syrup and 4·5 ± 0·43 h for the tablets. There were no significant differences in C_{max} values. The overall mean (±s.e.m.) terminal phase half-life was 4·78 ± 0·59 h. Mean (±s.e.m.) areas under the concentration time curves from 0 to infinity (AUC_∞) were 11·0 ± 1·99 ng h^{-1} ml^{-1} and 7·67 ± 1·05 ng h^{-1} ml^{-1} for syrup and tablets, respectively. The mean relative bioavailability for the tablets was approximately 70% with respect to the syrup.

Trimeprazine is an antipruritic and antihistaminic phenothiazine drug for which there is little published pharmacokinetic information. Johnson & Masters (1962) reported total radioactivity measurements in blood of animals given [^{35}S]-labelled drug. Heimlich et al (1961) reported the evaluation of an oral sustained release preparation of trimeprazine using a method that was not specific for the unchanged drug. A gas chromatographic method with nitrogen–phosphorus detection reported by Cailleux et al (1981) measured toxic levels of the drug.

Trimeprazine, in common with other phenothiazines, probably has a high apparent volume of distribution. Quantitation of such compounds was difficult until a high pressure liquid chromatographic (HPLC) method with electrochemical detection, developed by Curry & Brown (1981a) and Curry et al (1982), allowed determination of the drug in plasma or whole blood after single 5 mg oral doses as tablets or in syrup. McKay et al (1982) have used similar methodology. We have used the HPLC assay to estimate the drug in body fluids. We have also determined the stability, partition coefficient, plasma protein binding, and red blood cell distribution of the drug and estimated its relative bioavailability from tablets after single doses, using trimeprazine syrup as the standard dose form.

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MATERIALS AND METHODS

Materials

Trimeprazine powder (Lot 1–OTR (2), SK&F5277-F), trimeprazine tartrate syrup (USP), trimeprazine tablets (USP) (the marketed SK&F product) and trimeprazine tablets (USP) (an unmarketed equivalent) were from the Smith, Kline and French Company. Promazine and imipramine, used as chromatographic internal standards, were obtained as gifts from their respective manufacturers. Pesticide-grade n-hexane and HPLC-grade acetonitrile were purchased from Fisher Scientific (Pittsburg, PA, USA). All other chemicals were analytical grade and were purchased from Fisher Scientific. Buffer solutions were prepared to USP XX formulae.

Assay in whole blood

This was essentially as described previously (Curry & Brown 1981a, b; Curry et al 1982). The mean overall recovery ±s.d. at 0·5, 1, 2 and 5 ng ml^{-1} was 89·3 ±

11·0%. Retention times for trimeprazine and imipramine (internal standard) were 4·4 and 6·8 min, respectively. The within-day coefficient of variation was 3·4% for concentrations from 0·5 to 5 ng ml^{-1} ($n = 5$). Between-day coefficient of variation values varied from 11·8 to 78·2% (see Table 1) so daily standardization with spiked blood was conducted. Whole blood, rather than plasma was used, because the drug has high lipophilicity and low concentrations in body fluids.

Table 1. The within-day and between-day coefficients of variation and the percentage recovery in HPLC estimation of trimeprazine added to whole blood.

	Concn (ng ml^{-1})	Number of samples	CV %	% recovery (mean ± s.d.)
Within-day	0·5	5	1·39	—
	1	5	3·38	—
	2	5	2·77	—
	5	5	1·52	—
Between-day	1	5	78·2	—
	5	5	21·0	—
	10	5	11·8	—
Recovery	0·5	4	—	82 ± 2·8
	1	4	—	78 ± 8·5
	2	4	—	95·6 ± 6·2
	5	4	—	101 ± 0·5
				$\bar{X} = 89 \pm 11$

Apparatus

A Varian 5000 liquid chromatograph (Varian, Palo Alto, CA, USA) with a Valco injection valve and a Varian Micropak CN (10 μm) column were used with a glassy carbon electrochemical detector (Bioanalytical Systems, Lafayette, IN, USA) employing an LC-4A controller, and a silver–silver chloride reference electrode (R-1). The potential was set at +0·9 V. Detector response was recorded using both 1 and 10 mV strip chart recorders (LDC) and a CDS 111 integrator (Varian). The mobile phase was 90% acetonitrile and 10% 0·1 M ammonium acetate solution; the flow rate was 2·0 ml min^{-1}.

Partition studies as a function of pH

An aliquot (50 μl) of a stock solution of drug (100 μg ml^{-1}), as the tartrate, was dissolved in 5·00 ml of various buffer solutions to prepare solutions of $1{\cdot}338 \times 10^{-6}$ M base. Hexane (15 ml) was added to each buffer solution in a 30 ml extraction tube and extracted for 60 min. Aliquots of the 5 ml extracts were evaporated to dryness and redissolved in 100 μl of mobile phase containing 10 μg ml^{-1} promazine; 50 μl was chromatographed. A sample (50 μl) of stock solution ($1{\cdot}388 \times 10^{-6}$ M trimeprazine before extraction) was evaporated to dryness and redissolved in 100 μl of mobile phase containing 10 μl ml^{-1} promazine; 50 μl was chromatographed.

Extent of protein binding determined by equilibrium dialysis

One ml samples of human blood bank plasma were spiked with 968 or 1875 ng ml^{-1} trimeprazine, after being separated from whole blood by centrifugation, and then placed in knotted dialysis sacs (1·2 in flat width, Union Carbide Corp., Chicago, Illinois, presoaked overnight in the buffer used). Dialysis was in sealed vessels against 10·0 ml isotonic pH 7·4 phosphate buffer (1·9 g KH_2PO_4, 8·1 g Na_2HPO_4, and 4·11 g NaCl, made up to 1000 ml with H_2O, ionic strength 0·71) at 25 °C. Controls were run at both concentrations with sacs containing only 1 ml of buffer (no plasma) to check for any loss of drug during dialysis. At least four replications were made.

As a 'volume shift' occurs during equilibrium dialysis (Abel et al 1979; Tozer et al 1983) the fraction unbound was calculated using both traditional methods (eqn 1) and a new equation (eqn 2), recently published by Hu & Curry (1985); equation 2 allows for volume shift and drug losses (Aloss) during dialysis,

$$Fu' = C/Cp \qquad (1)$$

In this, C and Cp are the buffer and plasma concentrations, respectively. Both were measured after dialysis. However,

$$Fu = C\,Vp/(Ao - Aloss\,C\,Vb) \qquad (2)$$

where Vp and Vb are the plasma and buffer volume before dialysis, and Ao is the amount of drug in plasma before dialysis. The calculated volume shift (V′) during dialysis, according to equation 3, and the percent error of unbound fraction were also calculated:

$$V' = (Ao - C\,Vb - Cp\,Vp)/(Cp - C) \qquad (3)$$

Plasma/red blood cell concentration ratio, and red blood cell/plasma distribution coefficient

Since trimeprazine has relatively high lipophilicity, it seemed likely that a distribution between red blood cells (rbc) and plasma would occur. To analyse for this, the plasma/rbc (P/C) concentration ratio and rbc/plasma distribution coefficient were determined. Heparinized blood (120 ml) was divided into four parts: 72 ml was used for the study of rbc uptake of the drug, 10 ml was for measurements to give a calibration curve, 20 ml was used to study protein

binding (the red blood cells after centrifugation being used for calibration), and 18 ml was centrifuged and then used for measurements to give a plasma calibration curve. The 72 ml whole blood was equally divided among 36 tubes before drug was added. Final concentrations of 968 and 1875 ng ml^{-1} drug were achieved by addition of different volumes of trimeprazine stock solution. All tubes were incubated at 37 °C with shaking (0·5 Hz). Three tubes of each concentration were removed and centrifuged 0, 5, 15 and 30 min after drug was added. Both plasma and red blood cells were assayed. The ratio (P/C) was estimated from the plasma and rbc concentrations at various times. The rbc plasma distribution coefficient (D) was determined according to the equation:

$$D = [Arbc]/([Ap]\,Fu) \quad (4)$$

where [Arbc] and [Ap] are the concentrations of drug in red blood cells and plasma, respectively.

Bioavailability study

Six healthy, male, non-smoking volunteers, 20–40 years old, conforming to the desired height–weight range in the Metropolitan Life Insurance Company tables participated with informed consent in a study approved by the local institutional review board. The volunteers had a physical examination, and standard laboratory determinations (SMAC-25) were performed on control blood samples on each study day. No drugs were taken for at least 2 weeks before or during the study and no vitamin supplements were taken for the last 48 h before dosing. The morning doses of syrup or tablets (5 mg trimeprazine) were given with 240 ml of water following an overnight fast. Fluid (200 ml lemon flavoured soft drink) was given 2 h after dosing. The study used a randomized three-way crossover design. At least two weeks elapsed between treatments. Samples (10 ml) of heparinized blood were drawn before each dose, and at 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h after each dose; they were stored at −20 °C until analysed. The data were analysed by analysis of variance (ANOVA), the Multiple Range Test (MRT) which uses the least squared difference, and Newman Keul's MRT.

RESULTS AND DISCUSSION

Stability of trimeprazine during storage and analysis

The drug (dissolved in the mobile phase) was stable for 6 months when stored at −20 °C, and 3–5 months at 60 °C. In whole blood, the drug was stable for at least 24 h at 4 °C, and at 25 and 37 °C it was stable for at least 1 h.

Partition studies as a function of pH

The apparent hexane–water partition coefficient K was calculated from the observed peak height ratios from the HPLC analysis, in which the peak height ratios were shown to be directly proportional to concentration, by:

$$K = \frac{PHRo}{PHRt - PHRo} \cdot \frac{V_{aq}}{V_{org}} \quad (5)$$

where PHRt and PHRo are the peak height ratios for aqueous phase before extraction and organic phase after extraction, respectively, and V_{aq} and V_{org} are the respective volumes of the buffer and hexane solutions. Table 2 lists the apparent partition coefficients of trimeprazine at various pH values. Fig. 1 shows the respective percentages extracted by hexane from buffer solutions containing 1 and 0·6 μg ml^{-1} drug; the percentage extracted into the organic phase can be seen to be lower when pH values were higher than 10·54. This decline of the percentage extraction from strong alkaline solutions can be explained by decomposition of trimeprazine. This is supported by the rapid decomposition of 1 μg ml^{-1} trimeprazine solutions at pH = 12·64 and 90 °C. However, the drug was stable in 0·2 M potassium biphthalate buffer (pH = 4), at 90 °C. It seems that

Table 2. The apparent partition coefficients of trimeprazine (1 μg ml^{-1}) in 5 ml of various pH buffer solutions and 15 ml hexane.

pH	Apparent partition coefficient (K′)
4·82	1·50
6·00	4·53
7·00	7·53
8·20	8·58
9·30	16·28
10·54	129·40

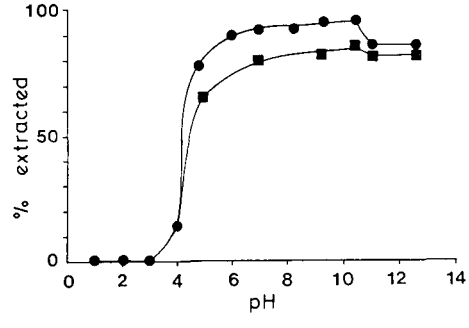

FIG. 1. Percent extraction of trimeprazine into hexane as a function of pH at two concentrations, (●), 1 μg ml^{-1}; (■), 0·6 μg ml^{-1}.

trimeprazine and its decomposition product reached equilibrium quickly, since there was no significant change in spectra after 1 h of shaking at pH <10·54.

Extent of protein-binding by using equilibrium dialysis

The equilibrium of unbound drug between plasma and buffer was found by preliminary experiments to be reached at 21·7 h. The percentage of drug not bound to protein was calculated with and without correction for volume shift using equations 1 and 2 (see Table 3). The true values of unbound fraction obtained were similar to the unbound fraction of chlorpromazine found by Curry (1970). The unbound fraction derived without consideration of volume shift had an error of 114% for trimeprazine (see Table 3); the average value for volume shift was 1·42 ml. The average recovery, estimated using controls, is listed in Table 3. The volume shift problem in equilibrium dialysis was discussed in detail elsewhere (Curry & Hu 1984; Hu & Curry (1985).

Plasma/rbc concentration ratio, and rbc/plasma distribution coefficient

The equilibrium distribution of trimeprazine between red blood cells and plasma was reached within 5 min of the drug being added. A kinetic plot of the red blood cell/plasma distribution gave mean values of P/C ratio and rbc/plasma distribution coefficient of 1·17 ± 0·26 and 8·65 ± 1·57, respectively. The detailed data are shown in Table 4.

Bioavailability study

The means of the blood concentrations obtained with the syrup and the commercial tablet are shown in Table 5. No 48 h sample contained any trimeprazine. These data imply that trimeprazine in the syrup may have had greater bioavailability than trimeprazine in the tablets. The mean (±s.e.m.) areas under the curve (units ng h^{-1} ml^{-1}) from time zero to the last time point of positive detecton of trimeprazine were 7·87 ± 2·11 (syrup) and 4·15 ± 0·76 (tablet). The mean areas under the curve to infinity were 11·00 ± 1·99 (syrup) and 7·67 ± 1·05 (tablet). The

Table 3. Percent of unbound trimeprazine in plasma by equilibrium dialysis (mean ± s.d.)

Trimeprazine concentration before dialysis (μg ml^{-1})	Plasma concn after dialysis (μg ml^{-1})	Buffer concn (μg ml^{-1})	Fu[a] %	Fu′ %	E[b] %	V′ (ml)
0·968	0·240 ± 0·029	0·0534 ± 0·0031	9·74 ± 1·10	21·05 ± 2·84	115·7 ± 19·8	1·47 ± 0·28
1·875	0·452 ± 0·067	0·0864 ± 0·006	9·26 ± 1·25	19·23 ± 1·51	111·6 ± 41·3	1·38 ± 0·52
Overall mean (n = 8)	—	—	9·50 ± 1·12	20·10 ± 2·26	113·7 ± 30·0	1·42 ± 0·39

[a] The recovery figures of 0·968 and 1·875 μg ml^{-1} were taken into account in calculation of Fu.
[b] The percent error of unbound fraction was calculated from E = (Fu′ − Fu) × 100/Fu.

Table 4. Red blood cell (rbc) localization and plasma/cell ratios of trimeprazine in whole blood with 0·968 and 1·875 μg ml^{-1} concentrations.

Drug whole blood concn (μg ml^{-1})	t[a] (min)	Plasma concn (μg ml^{-1}) (mean ± s.d.)	Rbc concn (μg ml^{-1}) (mean ± s.d.)	P/C[b] (mean ± s.d.)	D[c] (mean ± s.d.)	% Recovery (mean ± s.d.)
0·968	0	1·050 ± 0·056	0·717 ± 0·036	1·514 ± 0·034	6·78 ± 0·15	93·9 ± 3·4
	5	0·819 ± 0·060	0·853 ± 0·11	0·945 ± 0·032	10·88 ± 3·7	85·5 ± 9·6
	15	1·050 ± 0·10	0·750 ± 0·14	0·750 ± 0·02	7·26 ± 0·12	94·2 ± 3·7
	30	0·966 ± 0·18	0·792 ± 0·13	1·380 ± 0·08	7·45 ± 0·13	97·9 ± 2·3
	Mean ± s.d.	—	—	1·150 ± 0·36	8·09 ± 1·88	93·0 ± 5·2
1·875	0	1·830 ± 0·036	1·650 ± 0·18	1·130 ± 0·12	9·58 ± 1·42	93·8 ± 3·1
	5	1·920 ± 0·045	1·640 ± 0·20	1·180 ± 0·17	9·24 ± 1·36	95·3 ± 3·94
	15	1·620 ± 0·032	1·610 ± 0·12	1·020 ± 0·15	10·42 ± 1·47	87·0 ± 3·50
	30	1·760 ± 0·14	1·260 ± 0·014	1·430 ± 0·15	7·58 ± 0·81	82·3 ± 4·50
	Mean ± s.d.	—	—	1·190 ± 0·17	9·20 ± 1·19	89·6 ± 6·10
Overall mean ± s.d.		—	—	1·170 ± 0·26	8·65 ± 1·57	

[a] t is the time after drug added to whole blood.
[b] P/C ratio is the ratio of plasma concentration and red blood cell concentration after equilibrium is reached.
[c] D is red blood cell distribution coefficient. Fu = 0·0974, and 0·0926 for 0·368 and 1·875 μg ml^{-1} drug, respectively. Number of replications is three.

Table 5. Trimeprazine concentrations in blood of six fasting volunteers after single 5 mg oral doses (mean ± s.e.m.).

Time after dose (h)	Syrup	Tablet
0	0	0
0·5	0	0
1	0·21 + 0·13	0·08 + 0·07
2	1·60 ± 0·94	0·16 ± 0·10
3	2·12 ± 0·93	0·68 ± 0·13
4	1·06 ± 0·18	0·82 ± 0·15
5	0·61 ± 0·17	0·64 ± 0·22
6	0·54 ± 0·22	0·66 ± 0·14
8	0·35 ± 0·15	0·39 ± 0·10
10	0·22 ± 0·15	0
12	0·15 ± 0·09	0
24	0·08 ± 0·08	0

overall mean (±s.e.m.) terminal phase half-life of trimeprazine was 4·78 ± 0·59 h (rate constant 0·145 ± 0·028 h^{-1}). The mean peak blood concentrations were 2·34 ± 0·9 ng ml^{-1} (syrup) and 0·95 ± 0·13 ng ml^{-1} (tablet). The mean times to maximum concentration were 3·50 ± 0·22 h (syrup) and 4·50 ± 0·43 (tablet). There were significant differences in area under the curve, mean peak and mean time to concentration maxima data. The investigation involved three treatments, but the analysis above involved only the commercial syrup and commercial tablets. There were in fact no significant differences between the two tablets. There was no evidence of an order effect in the experiment, so no evidence of one treatment affecting the results of another. Our primary purpose was the comparison of tablets and syrup. The mean bioavailability of commercial tablets was apparently 70% of that of the syrup.

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