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BIOEQUIVALENCY AND DOSE PROPORTIONALITY OF THREE TABLETED PROMETHAZINE PRODUCTS

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ABSTRACT

Data from a five-way crossover study in human subjects using four talented promethazine products and a promethazine solution are presented. All products were administered as a single oral dose. The five objectives of the study were to investigate bioequivalency, to estimate dose proportionality at two dose levels, to establish validity of a reference production solution for future bioequivalency studies, to estimate intersubject variation, and to compare bioavailability/tablet dissolution data. Blood samples were collected at given intervals over a 24-hour period and analysed for promethazine using an HPLC technique. Pharmacokinetic parameters were calculated using standard procedures and a two-way analysis of variance (ANOVAR) was used to assess whether the differences were statistically significant. The $AUC_{0\rightarrow\infty}$ data from the ANOVAR analysis showed that the 50 mg innovator and generic products and the 50 mg solution were not significantly different. However, the innovator product had a significantly lower C_{max} and longer t_{max} than the solution. The generic product did not differ significantly from the solution. Promethazine was found to exhibit linear dose proportionality in the range and product studied. Intersubject variation was high for all parameters (23 to 63 per cent) and the *in vivo* and *in vitro* data showed a positive relationship.

KEY WORDS Promethazine Single dose Bioequivalence Dose proportionality Dissolution Pharmacokinetic parameters

INTRODUCTION

Promethazine is a widely used antihistaminic, antiemetic, and sedative drug. The pharmacokinetic properties of this drug have been reported in man following oral, intravenous, intramuscular, and rectal administration[1–7] and in animals following oral, intravenous, intramuscular, and hepatic portal vein

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administration.[8,9] The present study was undertaken in order to compare the pharmacokinetics of different currently marketed promethazine products after oral administration. Dose proportionality of promethazine in the form of an oral solution has been reported but not for promethazine tablets. Therefore, a two-level dose proportionality study also was included in the protocol.

The main objectives of our study were as follows:

1. to resolve any issues concerning bioequivalency problems with different promethazine dosage forms;
2. to investigate the dose proportionality of tableted promethazine at two dose levels;
3. to confirm the adequacy of a reference product solution for any necessary future *in vivo* studies.
4. to assess the extent of intersubject variability in pharmacokinetic parameters obtained with different dosage forms of promethazine;
5. to generate *in vivo* data which may prove useful in the establishment of a relationship between *in vivo* dissolution parameters and bioavailability.

MATERIALS AND METHODS

Fifteen normal healthy male adults aged between 18 and 32 years and weighing between 62.1 and 90.1 kg took part in the study. They were all within 10 per cent of their ideal body weight for their age, height, and body frame.[10] The study protocol was approved by the UGA Institutional Review Board and the FDA Research Involving Human Subjects Committee. The protocol was explained in detail to all the subjects and each signed a consent form before participating in the study.

The study utilized a five-way crossover design shown in Table 1. A placebo dose also was included for human response measurements which are presented elsewhere.[11] The promethazine tablets were administered orally with 240 ml of cold water. The oral solution (200 ml containing 0·25 mg ml^{-1} of promethazine hydrochloride in 2·5 per cent v/v of 95 per cent ethanol/distilled water) was administered followed by a rinse with 40 ml of cold water.

A 21-gauge butterfly catheter was inserted into a forearm vein of each subject and kept patent with heparin (10 USP units of heparin per millilitre of physiological saline). Ten-millilitre blood samples were collected via the catheter, prior to dosing (0 h) and at the following times after dosing: 0·5, 1·0, 1·5, 2·0, 3·0, 4·0, 6·0, 8·0, 10, 12, and 24 h. The last sample was collected by venipuncture.

The blood samples were collected in silanized 10 ml test tubes containing 150 USP units of heparin, gently shaken and centrifuged at 2000 rev min^{-1} for

Table 1. Dose administration sequence for promethazine products

Group*	Week				
	1	2	3	4	5
1	A	E	B	D	C
2	B	A	C	E	D
3	C	B	D	A	E
4	D	C	E	B	A
5	E	D	A	C	B

*Each group was composed of 3 subjects randomly assigned.
A = 50 mg solution.
B = Cord 50 mg tablet (Generic, Cord Laboratories, Broomfield, Colorado, Lot 50715).
C = Wyeth 50 mg tablet (Innovator, Wyeth Laboratories, Philadelphia, PA, Lot 1821148).
D = Wyeth 25 mg tablet (Lot 1821448).
E = Placebo tablet (supplied by the University of Georgia, Athens, GA 30602).

10 min. The plasma was separated and stored in silazined glass vials at −20° until analysis.

Subjects refrained from any medications 7 days before and 3 days after each study day. Subjects fasted for 8 h before each dose was administered and for 4 h thereafter. Lunch and dinner were provided 4 and 8 h, respectively, after dosage. Subjects refrained from any strenuous physical activities on each study day and were retained at the study centre for 12 h following dose administration.

The analytical procedure used to detect and quantitate promethazine levels was a modification of that reported by Wallace *et al.*[4] The HPLC system consisted of a pump (Model 110A, Beckman Instruments), a cyanopropyl column (4·6 mm × 150 mm, particle size 5 μm, Chromanetics Corp.), a fixed loop injector (100 μl, Rheodyne Model 7125), an electrochemical detector consisting of an amperometric controller (VA-Detector E611, Brinkman Instruments) and an electrochemical cell equipped with a silver–silver chloride reference electrode and a thin layer glass carbon working electrode (Model TL-5A, Bioanalytical Systems). The cell potential was set at +900 mV and an electronic recorder-integrator (Model 3390A, Hewlett-Packard) was used to record and integrate the chromatograms.

The mobile phase was 45:55 (v/v) acetonitrile : 0·02 M potassium dihydrogen phosphate, pH 6·0. The flow rate was 2·0 ml/min and the column was operated at room temperature.

The assay procedure was as follows. Two-millilitre plasma samples were placed in silazined 15 ml test tubes. Sodium chloride (about 0·6 g, Baker, reagent grade) followed by 100 μl of 0·1 M sodium hydroxide were added and the samples vortexed for 10 s. Ten millilitres of hexane (Baker, reagent grade) containing the internal standard trifluperazine hydrochloride (20 ng ml^{-1}) and 0·8 per cent n-butanol (Baker, reagent grade) was added and the mixture shaken gently for 15 min in a horizontal shaker. (Eberbach). Samples were centrifuged for 10 min at 2000 rev min^{-1}. The plasma layer was quick-frozen using a dry ice-acetone bath and the hexane layer was decanted into a silanized 10 ml test tube. The hexane was evaporated to dryness in an analytical evaporator (N-Evap, Organomotion Inc.) at 50° under a gentle stream of nitrogen. The residue was dissolved in 200 μl of mobile phase, vortexed for 60 s and 100 μl injected onto the column.

Calibration curves were prepared by spiking 2 ml aliquots of blank human plasma (Red Cross) with a promethazine standard solution (0·1 ng μl^{-1}) to give the following concentration: 0, 2, 5, 10, 20 and 40 3ng ml^{-1}. Linear regression analysis of drug concentration vs drug/IS peak height ratios gave slope and intercept data which was used to calculate the concentration of promethazine in individual plasma samples. A calibration curve was prepared daily for each set of subject samples.

The *in vitro* dissolution tests were of the same batch of tablets as administered to the subjects and were performed by the Food and Drug Administration (Center for Drug Analysis, St. Louis, Missouri), using the USP paddle procedure at 50 rev min^{-1} in distilled water.

RESULTS AND DISCUSSION

Chromatograms of samples from one subject before dosing, 1·0 h after dosing and plasma spiked with promethazine and internal standard are shown in Figure 1. There was no interference from endogenous compounds at the retention times that the drug and internal standard eluted. Typical calibration data for spiked plasma samples in the 2–40 ng ml^{-1} range for promethazine is shown in Table 2. The accuracy and precision at two different concentration levels are shown in Table 3.

The mean plasma profiles of the four promethazine products studied are shown in Figure 2. For clarity, the standard deviations (SD) are not shown. Plasma concentrations and corresponding standard deviations are presented in Table 4. The mean (± SD) area under the plasma concentration vs time curve from zero to infinity ($AUC_{0\to\infty}$), half life ($t_{½}$), elimination rate constant

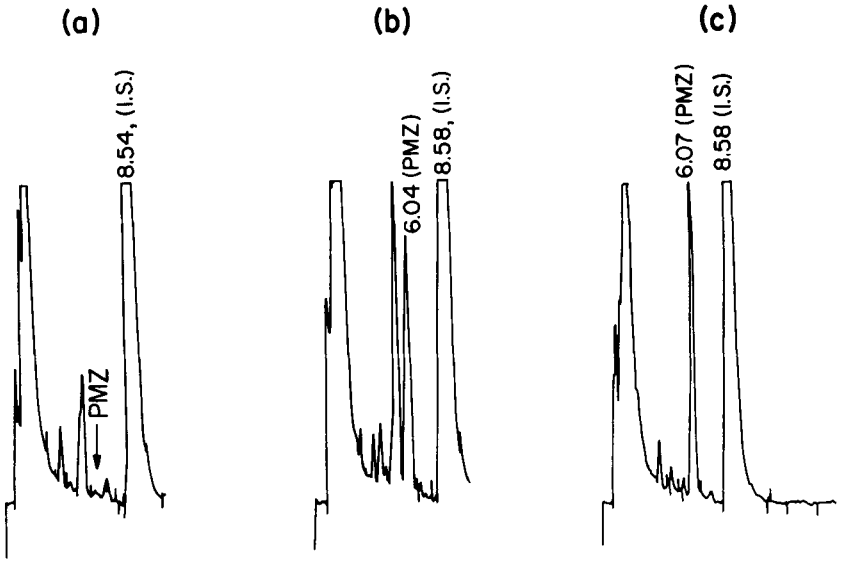

Figure 1. HPLC chromatograms obtained from extracts of (a) subject plasma at 0·0 h (i.e. before dosing); (b) subject plasma at 1·0 h after dosing with promethazine (PMZ); and (c) spiked plasma containing 10 ng ml^{-1} of promethazine and 100 ng ml^{-1} of trifluperazine hydrochloride as internal standard (I.S.)

Table 2. Typical calibration curve data for plasma samples spiked with promethazine (PMZ)

PMZ conc. (ng ml^{-1})	Pk. Ht. ratio (PMZ/IS)	Linear regression
2	0·0915	
		$n = 5$
5	0·2629	
		$r^* = 0{\cdot}9977$
10	0·4201	
		intercept = 0·0553
20	0·8495	
		slope = 0·0373
40	1·5243	

*Correlation coefficient.

(K_e), maximum plasma concentration attained (C_{max}), and time required to attain C_{max} (t_{max}) of the different products are presented in Table 5.

A two-way analysis of variance was used to assess whether the differences in the pharmacokinetic parameters of the four promethazine products when

Table 3. Assay of spiked plasma samples for promethazine

PMZ conc. added (ng ml^{-1})	PMZ conc. found (ng ml^{-1})	Per cent error*	RSD%†
8·0	8·69 ± 0·23	8·6	2·6
30·0	31·37 ± 1·37	4·6	4·4

* $\frac{\text{Conc. found} - \text{Conc. added}}{\text{Conc. added}} \times 100.$

†Relative standard deviation (SD/mean × 100).

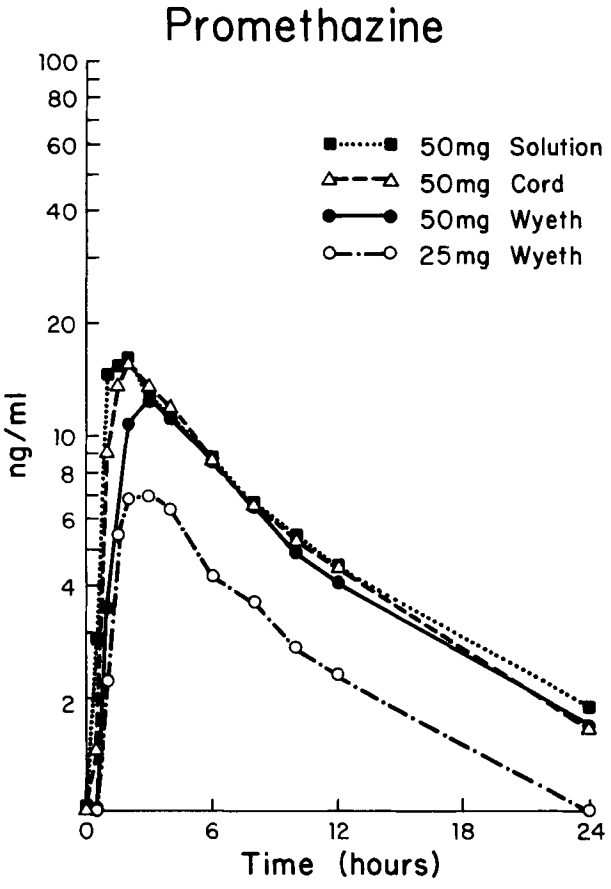

Figure 2. Mean (n = 15) plasma profiles for the four promethazine products

Table 4. Mean ($n = 15$) Plasma concentrations (ng ml^{-1}) of promethazine vs time for the promethazine (PMZ) products

Time after dosage (h)	Product			
	50 mg solution	50 mg generic tablet	50 mg innovator tablet	25 mg innovator tablet
0	–	–	–	–
0·5	2·86 (2·67)*	1·44 (2·82)	0·26 (0·75)	0·12 (0·45)
1	14·67 (13·70)	8·99 (8·77)	3·62 (3·05)	2·20 (1·76)
1·5	15·39 (10·39)	13·61 (9·98)	6·65 (4·15)	5·38 (4·26)
2	16·18 (9·84)	15·75 (9·10)	10·74 (3·67)	6·80 (4·42)
3	12·68 (6·88)	13·61 (6·55)	12·54 (6·22)	6·91 (3·42)
4	11·38 (6·75)	11·91 (5·32)	11·20 (4·42)	6·32 (2·90)
6	8·63 (4·20)	8·65 (4·35)	8·54 (3·04)	4·25 (2·00)
8	6·60 (3·54)	6·57 (2·85)	6·48 (2·43)	3·60 (1·53)
10	5·42 (3·09)	5·25 (2·50)	4·85 (1·66)	2·72 (1·27)
12	4·46 (3·30)	4·45 (2·95)	4·05 (2·07)	2·30 (1·35)
24	1·91 (1·75)	1·67 (1·75)	1·70 (1·64)	0·67 (0·94)

*Figures in parentheses are the corresponding standard deviations.

Table 5. Mean pharmacokinetic parameters for the promethazine products

Parameter	Product			
	50 mg solution	50 mg generic tablet	50 mg innovator tablet	25 mg innovator tablet
$AUC_{0\to\infty}$ (ng h ml^{-1})	143·13 (106·84)*	142·16 (106·20)	118·00 (62·13)	61·49 (37·95)
C_{max} (ng ml^{-1})	18·31 (12·56)	16·88 (8·69)	13·99 (5·72)	7·91 (4·15)
t_{max} (h)	1.83 (0·70)	2.40 (0·95)	3.00 (1·18)	3.07 (1·21)
$t_{1/2}$ (h)	6·21 (2·50)	6·09) (2·80)	5·89 (2·02)	5·82 (1·63)
K_e (h^{-1})	0·133 (0·066)	0·129 (0·043)	0·130 (0·044)	0·129 (0·042)

*Figures in parentheses are the corresponding standard deviations.
$AUC_{0\to\infty}$ = Area under the plasma concentration vs time curve from zero to infinity (calculated using trapezoidal rule).
$t_{1/2}$ = half-life (calculated from the slope, K_e, of the terminal phase of a plot of ln conc. vs time).
K_e = elimination rate constant.
C_{max} = maximum plasma concentration attained.
t_{max} = time taken to attain C_{max}.

Table 6. Statistical comparison (probability values, *t*-table) of the pharmacokinetic parameters of the four different products. Values $\leq 0{\cdot}05$ are considered statistically significant

Parameter	Solution vs generic-50	Solution vs innovator-50	Solution vs innovator-25	Generic-50 vs innovator-50	Generic-50 vs innovator-25	Innovator-50 vs innovator-25
AUC_0	N.S.*	N.S.	0·0001	N.S.	0·0001	0·0045
C_{max}	N.S.	0·0388	0·0001	N.S.	0·0001	0·0049
t_{max}	N.S.	0·0019	0·0011	N.S.	N.S.	N.S.
$t_{½}$	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
K_e	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

*Not significant.

compared against each other were statistically significant (Table 6). A p-value $\leq 0{\cdot}05$ was considered to indicate a statistically significant difference.

There were no significant differences in the $AUC_{0\to\infty}$ values between the 50 mg solution, 50 mg generic and 50 mg innovator products. However bioequivalence based on AUC values could not be established for these products because the statistical power of the ANOV test was only 23 per cent to detect a 20 per cent difference between the innovator product and the other 50 mg dosage forms. This lack of statistical power was unexpected, based on previous studies of intra- and intersubject variance of oral promethazine. It should be noted though that the innovator tablet gives a lower C_{max} when compared to the solution. This may be due to a slower rate of absorption since both the 50 and 25 mg innovator products have significantly longer t_{max} values when compared to the solution. As expected, the differences in $AUC_{0\to\infty}$ and C_{max} values were statistically significant when the 25 mg innovator product was compared to the other three 50 mg products. Values for the pharmacokinetic parameters obtained with the 50 mg oral solution are in agreement with those reported by Schwinghammer *et al.*[7] for a 50 mg syrup. They are not in agreement with values reported by Moolenaar *et al.*[3] This may be because the Moolenaar study had a much smaller number of subjects than the present one.

The innovator 25 and 50 mg tablets illustrate that doubling the dose doubles the $AUC_{0\to\infty}$. Therefore, in the range and product studied, a tableted promethazine dosage form shows linear dose proportionality. This result is in

Table 7. Intersubject variation of pharmacokinetic parameters

Parameter	Mean*	Standard deviation	Relative standard deviation (%)†
$AUC_{0\to\infty}$ ($ng\,h\,ml^{-1}$)	116·20	72·85	62·7
C_{max} ($ng\,ml^{-1}$)	14·27	7·11	49·8
t_{max} (h)	2·58	0·59	22·8
$t_{1/2}$ (h)	6·00	1·71	28·4
K_e (h^{-1})	0·13	0·03	23·6

*Mean of all (15) subjects over all (4) doses.
† $\frac{\text{Standard deviation}}{\text{Mean}} \times 100$.

Table 8. *In vitro* dissolution data* of the promethazine commercial tablets

Product	Dissolution time 15 min	30 min
Generic 50	89†	92
Innovator 50	80	96
Innovator 25	96	100

*The *in vitro* dissolution tests were performed by the FDA (CDA, St. Louis, Missouri) using the USP paddle procedure at 50 rev min^{-1} in water.
†Per cent of tablet dissolved. There was no statistically significant difference in dissolution among the product tested.

disagreement with that reported by Moolenaar *et al.*[3] The lack of dose proportionality reported by these workers may be due to the small sample size (six subjects) and the large variance noted in the earlier study.

The $t_{1/2}$ and K_e values of the four promethazine products were not significantly different from each other.

The promethazine solution can be used as a reference product for comparing $AUC_{0\rightarrow\infty}$, however, the C_{max} and t_{max} values for the innovator product showed a significant difference. Our results show that a tableted dosage form can emulate a solution reference product. It is interesting that the innovator product does not.

The extent of intersubject variation was estimated as follows. For each pharmacokinetic parameter a mean value for each subject was calculated which included all four products administered. A mean and standard deviation of these 15 mean subject values along with the relative standard deviation were calculated for each parameter. The data, as seen in Table 7, illustrate that the intersubject variation was high for all parameters particularly $AUC_{0\rightarrow\infty}$ and C_{max}. This variance is probably a result of the significant first-pass metabolism of promethazine previously reported in the literature.[1,2,6]

No significant difference was found in the dissolution rates of the 50 mg innovator and 50 mg generic products (Table 8). Since the $AUC_{0\rightarrow\infty}$ values of these products were also not significantly different, it can be said that the *in vitro* procedure assures satisfactory bioavailability.

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