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Relative Bioavailability of a Commercial Trifluoperazine Tablet Formulation using a Radioimmunoassay Technique

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Abstract □ The relative bioavailability of a new conventional tablet formulation (5 mg) of trifluoperazine dihydrochloride was studied in 24 healthy volunteers. Using a sensitive radioimmunoassay technique, plasma trifluoperazine concentrations were measured up until 24 h following ingestion of single 5-mg doses of trifluoperazine. The mean ± *SD* for the peak concentration (C_{max}), time to C_{max}, area under the curve from 0 to 24 h (AUC_0^{24}), and terminal elimination half-life following the administration of the test formulation were 2.15 ± 1.07 ng/mL, 4.10 ± 1.38 h, 21.04 ± 11.92 ng·h/mL, and 9.5 ± 7 h, respectively. Following the ingestion of the original trifluoperazine tablet formulation (5 mg) these same parameters were estimated to be 1.92 ± 0.88 ng/mL, 4.02 ± 1.10 h, 18.03 ± 10.11 ng·h/mL, and 9.3 ± 7 h, respectively. Large intersubject variations in C_{max} and AUC_0^{24} were observed. The relative bioavailability of the test formulation was calculated to be 106.5 ± 25.5%.

Keyphrases □ Trifluoperazine—relative bioavailability, commercial tablet formulation, RIA technique □ Bioavailability—relative, commercial trifluoperazine tablet formulation, RIA technique □ Radioimmunoassay—relative bioavailability, commercial trifluoperazine tablet formulation

Trifluoperazine is an orally administered phenothiazine antipsychotic agent that has been in clinical use since 1958. Bioavailability studies of this drug have not been hitherto reported for a number of reasons. Trifluoperazine undergoes extensive metabolism to many metabolites which are formed from attack on both the phenothiazine ring and the side chain (1). Trifluoperazine is also known to undergo pronounced presystemic biotransformation in animals following oral administration (2). Phenothiazine drugs in general undergo significant first-pass effects in humans which contribute to large intersubject variability (3–5). Therefore, bioavailability studies require sensitive and specific analytical procedures. Recent analytical methods for trifluoperazine in plasma include GC–NPD (nitrogen-phosphorus detection) (6–8), GC–MS (9, 10), and radioimmunoassay (RIA) (11). Of these the GC–MS and RIA procedures have been used in single-dose pilot studies, where it was found that the bioequivalency parameters as determined by RIA were similar to those determined using GC–MS (12).

This study describes the estimation of the bioavailability of a new conventional trifluoperazine tablet formulation (5 mg)[1] relative to the original product[2]. Following single oral doses of 5 mg, the plasma concentration–time profiles of trifluoperazine were examined up to 24 h using RIA, which is sensitive to 0.25 ng/mL using a 200-μL plasma sample (11).

Table I—*In Vitro* Tablet Test Results

Test	Method	Test Product	Reference Product
Assay, mg (% potency)	USP XX	4.88 (97.6%)	5.23 (104.6%)
Disintegration[a], min	USP XX	5.5	7.0
Content uniformity, % (*RSD*, %)	USP XX	95.7 (3.04)	100.5 (1.69)
Dissolution, % (*RSD*, %)	(Gastric test solution, no enzyme)	100.7 (1.50)	80.7 (30.13)

[a] Gastric test solution.

EXPERIMENTAL

Tablet samples from production lots of two formulations of trifluoperazine were assigned as test[1] and reference[2]; standard *in vitro* tests were performed on both tablet formulations. The dissolution test was carried out on six individual tablets using apparatus 1, as described (13). The basket was rotated at 50 rpm, and the dissolution medium (900 mL) was 1% HCl (v/v) at 37 ± 0.5°C. At the end of 30 min, a suitable portion of the dissolution fluid was filtered. After discarding the first 20 mL of the filtrate, the absorbance of the standard and dissolution test preparations were determined in 1-cm cells at 255 (the wavelength of maximum absorbance) and 278 nm (the wavelength of minimum absorbance) using 1% HCl (v/v) as the blank.

Twenty-four healthy adult male volunteers, from whom written informed consent was obtained, were included in this study. With one exception, all were nonsmokers. The fitness of each subject was assessed by an independent physician who conducted complete physical examinations, reviewed medical histories and the results of clinical laboratory tests (hematology, SMA 12 biochemistry screen, and urinalysis), and monitored the health of the subjects throughout the study period. All subjects were drug free 30 d prior to the study and were asked to refrain from taking any drugs during the study, including abstaining from alcohol for 24 h, prior to and 24 h following each dose. The subjects were assigned randomly to receive the test or reference formulation for the first dose

[1] Trifluoperazine hydrochloride, lot #79-082, Cord Laboratories, Ltd., Broomfield, Colo.

[2] Stelazine, lot #2129S06, Smith Kline & French Ltd., Philadelphia, Pa.

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Table II—Mean Plasma Trifluoperazine Concentrations by Formulation

Formulation	Trifluoperazine Concentration, ng/mL									
	0.5 h	1.0 h	1.5 h	2.0 h	3.0 h	4.5 h	6.0 h	8.0 h	12 h	24 h
Test	0.18	0.55	1.04	1.43	1.93	1.89	1.36	1.03	0.84	0.35
(*SD*)	(0.23)	(0.65)	(0.72)	(0.83)	(1.06)	(1.03)	(0.86)	(0.61)	(0.41)	(0.35)
Reference	0.16	0.52	0.91	1.25	1.69	1.81	1.30	0.98	0.81	0.33
(*SD*)	(0.19)	(0.38)	(0.72)	(0.77)	(0.80)	(0.92)	(0.73)	(0.50)	(0.43)	(0.31)

Table III—Mean Values of Bioavailability Parameters of Trifluoperazine Formulations

Formulation	AUC_0^{24}, ng·h/mL	ln AUC_0^{24}, ng·h/mL	C_{max}, ng/mL	ln C_{max}	t_{max}, h	AUC_0^{24} Ratios, %[a]	C_{max} Ratios, %[a]	K_{el} (3–12 h), h^{-1}	K_{el} (12–24 h), h^{-1}
Test	20.54	2.86	2.10	0.61	4.10	106.54	108.39	0.1023	0.0729
(*SD*)	(11.63)	(0.61)	(1.04)	(0.55)	(1.38)	(25.46)	(25.50)	(0.0486)	(0.0330)
Reference	19.27	2.83	1.92	0.56	4.02	—	—	0.1013	0.0748
(*SD*)	(10.58)	(0.52)	(0.87)	(0.44)	(1.10)	—	—	(0.0455)	(0.0391)

[a] Test/reference formulation.

and then the respective alternate formulation for the second dose. There was a 2-week interval between treatments (balanced complete block). Single oral doses of trifluoperazine (5 mg) were administered with 100 mL of water to overnight-fasted subjects. Fluid and food intake were controlled for 4 h following each dose with a carbonated lemon-lime beverage (280 mL) and a standard lunch provided at 1.5 and 4 h, respectively. Blood samples were obtained by venipuncture immediately before and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.5, 6.0, 8.0, 12, and 24 h after administration. During sampling, care was taken to prevent the blood from coming in contact with the rubber stopper of the heparinized evacuated sampling tubes[3] (14). The blood samples were centrifuged, and the separated coded plasma samples were stored at −20°C until analysis.

Three variables for bioequivalency, *i.e.*, area under the plasma concentration–time curve (AUC_0^{24}), maximum observed concentration (C_{max}), and time to C_{max} (t_{max}) were examined in this study. Plasma concentrations of trifluoperazine appeared to decline biexponentially, and elimination rate constants for trifluoperazine were calculated for the two phases (3–12 h and 12–24 h) employing least-squares linear regression analysis of the semilogarithmic data. The AUC_0^{24} was estimated using the trapezoidal rule to C_{max}, followed by the logarithmic trapezoidal rule (15) to 24 h. C_{max} and t_{max} were obtained directly from the raw data. The three-way analysis of variance of AUC_0^{24}, C_{max}, and t_{max} was carried out on the raw data. This analysis was also performed on natural logarithmic transformation of AUC_0^{24} and C_{max}. The subject effect, period effect, and the formulation effect were taken into account in each analysis. All results are reported as mean ± *SD* unless otherwise stated.

RESULTS AND DISCUSSION

Table I shows the results of the *in vitro* data for both the test and reference formulations of trifluoperazine. The assay results demonstrated that the test formulation was 97.6% and the reference formulation 104.6% of the label strength (5 mg).

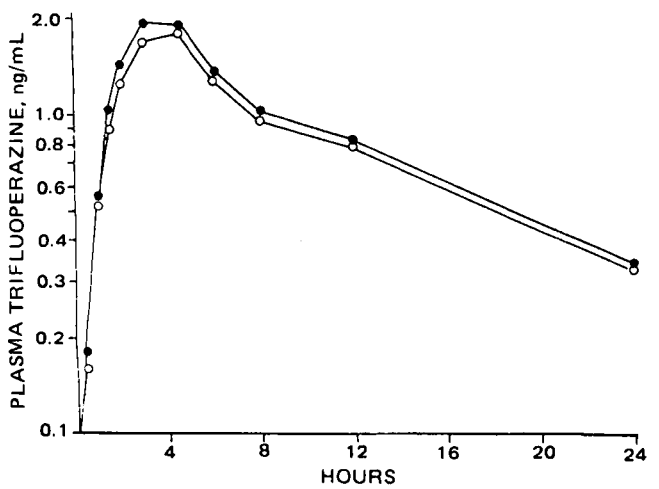

Figure 1—*Mean plasma trifluoperazine concentrations for test (●) and reference (○) formulations.*

[3] Vacutainer B. D., Becton, Dickinson & Co., Mississauga, Ont.

Figure 1 depicts the semilogarithmic mean plasma concentration–time profiles of trifluoperazine for the two formulations. The multicompartmental behavior of trifluoperazine and the limited number of correctly timed samples made it difficult to obtain meaningful estimates of the absorption, distribution, and elimination rate constants (16, 17).

Table II shows the mean plasma concentration–time data for the 24 subjects. Values reported <0.25 ng/mL are only estimates, as they fall below assay sensitivity (11). All other bioavailability data is presented in Table III. The AUC_0^{24} ranges for reference and test formulations were 6.3–53.3 and 3.9–55.5 ng·h/mL, respectively. The C_{max} values for reference and test formulations ranged from 0.67 to 4.28 and 0.43 to 4.64 ng/mL, respectively. The t_{max} ranged from 2 to 6 h for the reference formulation and from 2 to 8 h for the test formulation. The AUC_0^{24} (8- to 14-fold) and C_{max} (6- to 10-fold) ranges clearly demonstrated the large intersubject variability typical of the oral phenothiazine drugs (3–5), which is shown here for the first time for single doses of trifluoperazine.

The AUC_0^{24} and C_{max} ratios of test–reference formulation were 106.5 ± 25.5 and 108.4 ± 25.5, respectively, which demonstrates that the test product has acceptable relative bioavailability. This is further supported by the fact that 18/24 and 17/24 of the subjects who participated in this study had relative bioavailabilities of 100 ± 25% in terms of AUC and C_{max} ratios, respectively. This clearly establishes the bioequivalency of the test formulation.

Table IV presents the results of the analysis of variance. There are no statistically significant differences in the formulations in terms of AUC_0^{24}, ln AUC_0^{24}, and ln C_{max}, while there is statistically significant difference in C_{max}. However, this latter difference may not be of significance in the clinical situation. There is a subject effect on all of the parameters examined, and a period effect on AUC_0^{24}. The 95% confidence intervals for the test and reference formulations are summarized in Table V. It is clear that there is no detectable difference between the test and reference formulations for all the parameters examined. The narrow and overlapping confidence intervals for AUC_0^{24} and C_{max} further establish the bioequivalency of the test formulation as compared with the reference.

After reaching C_{max}, trifluoperazine plasma concentrations decline at least in a biphasic manner. The apparent terminal half-lives were unfortunately estimated from only two data points in each case (12 and 24 h). The mean values for apparent terminal half-lives for test and reference were 9.5 ± 7 and 9.3 ± 7 h, respectively. Information concerning trifluoperazine plasma concentrations in humans is scarce because of the lack of sensitive assay methods. In a patient under chronic treatment at various times with daily doses of 15, 30, and 80 mg, Curry *et al.* (18) was able to measure plasma trifluoperazine concentrations only at the 80-

Table IV—Three-way Analysis of Variance

	α-Values				
	AUC_0^{24}	ln AUC_0^{24}	C_{max}	ln C_{max}	t_{max}
Formulation	0.057	0.303	0.045*	0.219	0.606
Period	0.009[a]	0.010[a]	0.144	0.174	0.276
Subject	0.000[a]	0.000[a]	0.000*	0.000*	0.000[a]

[a] Significantly different from 100% at $\alpha = 0.05$, based on two-tailed t test.

Table V—Average Values of Bioavailability Parameters with 95% Confidence Intervals [a]

Formulation	AUC_0^{24}, ng-h/mL	C_{max}, ng/mL	t_{max}, h	Relative Bioavailability, %[b] AUC_0^{24}	C_{max}
Test	17.46	1.84	4.10	103.63	105.65
Confidence Intervals	(16.17–18.75)	(0.58–3.10)	(3.52–4.68)	(93.52–114.82)	(95.63–116.72)
Reference	16.95	1.75	4.02	—	—
Confidence Intervals	(15.70–18.20)	(0.55–2.95)	(3.56–4.48)	—	—

[a] These mean values are based on natural logarithm transformed data. [b] Test/reference formulations.

mg/d dose level. In the present study trifluoperazine plasma concentrations were followed for 24 h following a single 5-mg dose. The characteristic phenothiazine multicomponent elimination was demonstrated for trifluoperazine. The C_{max} to 12-h phase is clearly distinct from the 12–24-h elimination following cessation of dosing. Animal studies have indicated that trifluoperazine, like other phenothiazine antipsychotics, appears to be extensively biotransformed presystemically (2); most of this may occur during first passage through the liver or in the gut. This may explain the large intersubject variability seen here with trifluoperazine, which is very characteristic of a drug with a high hepatic extraction ratio (19–21). Alteration in the activity of drug metabolizing enzymes by factors such as diet and smoking (22) may produce significant variations in the plasma concentrations of trifluoperazine following oral administration.

The present study demonstrated that trifluoperazine plasma concentrations can be monitored for as long as 24 h following the oral administration of single 5-mg doses of trifluoperazine. The measurement of these plasma concentrations has allowed us to successfully establish the relative bioavailability of a newly developed trifluoperazine formulation. These results suggest that the available analytical methodology should be able to monitor plasma concentrations in patients under chronic treatment with even low doses of trifluoperazine so that plasma concentration *versus* clinical response correlations can be investigated.

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