Radioimmunoassay for the Sulfoxide Metabolite of Trifluoperazine and Its Application to a Kinetic Study in Humans

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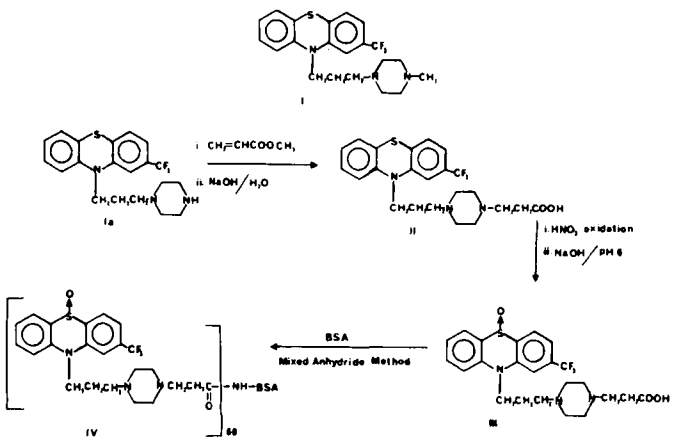
Abstract □ Antibodies were produced in rabbits immunized with 10-[[3-[4-(2-carboxyethyl)-1-piperazinyl]-propyl]]-2-trifluoromethyl-10*H*-phenothiazine sulfoxide–bovine serum albumin conjugate. The subsequently developed radioimmunoassay (RIA) procedure enables, for the first time, the quantitation of the sulfoxide metabolite of trifluoperazine in the plasma of humans after administration of therapeutic doses of trifluoperazine, in which 60 pg of the sulfoxide metabolite in 200 μL of plasma can be measured with a *CV* of <3%. Similar results were obtained by this assay with or without a benzene extraction step and also in the presence or absence of a large excess of trifluoperazine and suspected major metabolites of trifluoperazine. This RIA procedure, together with a previously developed RIA for trifluoperazine was used to directly determine plasma concentrations of trifluoperazine and its sulfoxide metabolite after administration of a single, low, oral dose of trifluoperazine to five healthy volunteers. The rapidly appearing, relatively high concentrations of the sulfoxide metabolite are indicative of presystemic sulfoxidation. The mean plasma elimination half-life for the sulfoxide metabolite of trifluoperazine was 5.8 ± 1.3 h.

Keyphrases □ Pharmacokinetics—trifluoperazine and its sulfoxide metabolite, quantitation by radioimmunoassay □ Radioimmunoassay—trifluoperazine and its sulfoxide metabolite, humans □ Trifluoperazine—pharmacokinetics, radioimmunoassay, sulfoxide metabolite

Phenothiazine antipsychotic agents are extensively metabolized into numerous metabolites, some of which may be of importance with regard to the therapeutic and/or toxic effects of drug treatment. For example, in the case of the sulfoxide metabolite of chlorpromazine, high plasma levels of the drug, which is reputed to be inactive, are associated with poor clinical response in schizophrenic patients under chronic treatment with chlorpromazine. Also, in the case of chlorpromazine, some workers have demonstrated that clinical response correlates better with plasma level ratios involving active drug–inactive drug (such as the 7-hydroxylated material plus chlorpromazine–sulfoxide metabolite) rather than with the steady-state levels of chlorpromazine (1–3). With the piperazine-type, phenothiazine, antipsychotic agent trifluoperazine (I; Scheme I), the importance in clinical therapy of the presumed major metabolites of this drug, including the sulfoxide which is reputed to be inactive, is unknown. The major reason for this is that suitable, sensitive, and specific analytical methods have not been available to measure metabolite concentrations in the plasma of patients under treatment. This slow development of analytical methods can be largely attributed to the reputed instability and adsorptive loss of phenothiazines in all stages of sample handling for analysis and the low plasma levels encountered due to the low doses of trifluoperazine given to patients. In fact, only recently were ultrasensitive analytical methods such as GC–MS (4, 5) and radioimmunoassay (RIA) (6, 7) reported for the quantitation of trifluoperazine in plasma. By these methods, trifluoperazine concentrations were quantitated in plasma after administration of low, single, oral doses of this drug. On the other hand, less sensitive techniques may not be adequate for trifluoperazine plasma level monitoring in most patients; for example, by a method based on HPLC with UV detection, measurement of trifluoperazine in plasma was obtained only while the patient was under chronic treatment with doses of trifluoperazine well above the recommended therapeutic dose (8).



Scheme I

The ultrasensitive biological method of RIA has the advantage over chemical methods of analysis such as GC–MS in that extraction of the biological sample may not be necessary and such methods are more amenable to routine clinical monitoring. In this report, a rapid, sensitive, and specific RIA for the sulfoxide metabolite of trifluoperazine is described. By using this method and a previously reported RIA for trifluoperazine (6), the concentrations of trifluoperazine and the sulfoxide metabolite were determined in plasma samples up to 24 h after the administration of a single 5-mg oral dose of trifluoperazine to five healthy volunteers.

EXPERIMENTAL SECTION

Materials—The sulfoxide, *N*-demethyl, and 7-hydroxy metabolites of trifluoperazine were synthesized in this laboratory by modified literature

Table I—Cross-Reactions [a] of Antiserum Against the Sulfoxide Metabolite of Trifluoperazine

Compound Tested	Cross-Reaction, %
Sulfoxide metabolite of trifluoperazine	100
Trifluoperazine	<1
Sulfone metabolite of trifluoperazine	8
N-Oxide metabolite of trifluoperazine	<1
7-Hydroxy metabolite of trifluoperazine	<1
N-Demethyl metabolite of trifluoperazine	<1
Ethylenediamine metabolite of trifluoperazine	<1
Sulfoxide metabolite of prochlorperazine	39
Sulfoxide metabolite of fluphenazine	62
Sulfoxide metabolite of perphenazine	77
Sulfoxide metabolite of chlorpromazine	<1
Ring sulfoxide metabolite of thioridazine	<1
Prochlorperazine	<1
N-Demethyl sulfoxide metabolite of trifluoperazine	55

[a] Cross-reactions were assessed by the criteria of Abraham (15).

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Table II—Determination by Direct Radioimmunoassay of the Sulfoxide Metabolite in Plasma Samples Spiked with a Fivefold Excess of Trifluoperazine or one of Its Metabolites [a]

Compound Added	Plasma Conc. of the Sulfoxide Metabolite of Trifluoperazine, ng/mL ± SD				
	n	1	2	3	5
Trifluoperazine	5	0.9 ± 0.3	1.8 ± 0.2	2.8 ± 0.2	5.1 ± 0.4
7-Hydroxy metabolite of trifluoperazine	3	1.2 ± 0.4	2.1 ± 0.2	2.9 ± 0.1	4.7 ± 0.1
N-Oxide metabolite of trifluoperazine	3	1.0 ± 0.3	2.1 ± 0.3	3.3 ± 0.1	5.0 ± 0.3
N-Demethyl metabolite of trifluoperazine	3	1.0 ± 0.1	2.0 ± 0.1	2.9 ± 0.3	4.9 ± 0.2

[a] At each standard curve concentration, none of the mean values found for samples containing added trifluoperazine or metabolites differed significantly ($p > 0.05$) from the standards containing the sulfoxide metabolite only determined in the same experiment.

Table III—Intra- and Interassay Variance of the Radioimmunoassay for Trifluoperazine

	Plasma Conc. of the Sulfoxide Metabolite of Trifluoperazine, ng/mL							
	10	7.5	5	3	2	1	0.5	0.3
Intra-assay variance[a]								
SD (B/B_0, %)	0.83	0.89	0.86	1.13	1.73	1.07	0.90	0.48
n	4	4	4	4	4	4	4	4
CV, %	2.5	2.3	1.8	1.9	2.5	1.3	1.0	0.5
Interassay variance[b]								
SD (B/B_0, %)	0.97	0.90	0.69	1.31	2.01	2.27	1.49	0.82
n	7	7	7	7	7	7	7	7
CV, %	3.0	2.3	1.4	2.1	2.7	2.7	1.6	0.8

[a] Intra-assay variance was calculated from assay readings of plasma standards obtained on a single day of analysis. [b] Interassay variance was calculated from assay readings of plasma standards obtained on four different days of analysis.

procedures (9, 10), whereas trifluoperazine dihydrochloride[1] and the other phenothiazines and their metabolites[2] were donated. Aromatic ring-labeled, tritiated trifluoperazine (12.8 Ci/mmol) was procured from a commercial source[3].

Liquid scintillation counting, in which a commercial cocktail was used[4], was performed in a counter equipped with automatic quench compensation[5]. All solvents were analytical grade. The dextran-coated charcoal suspension and the various buffers were prepared in this laboratory. TLC sheets precoated with silica gel without fluorescence indicator (250-xx thickness), used for tracer isolation, were obtained commercially[6]. All experimental procedures were carried out under subdued lighting.

Synthesis of the Immunogen (IV)[7]—To an aqueous solution (10 mL) of 10-[[3-[4-(2-carboxyethyl)-1-piperazinyl]-propyl]]-2-trifluoromethyl-10*H*-phenothiazine sulfoxide dihydrochloride (II) (1.1 g, 2 mmol) (11), was added concentrated HNO_3 in a dropwise manner. The mixture was left in the dark at room temperature for 2 h. During this time the mixture changed from a reddish-brown precipitate to a clear pale-yellow solution. The mixture was adjusted to pH 6.0 with 1 M NaOH solution and subsequently saturated with NaCl. The product was extracted into dichloromethane (5 × 20 mL) and dried over anhydrous sodium sulfate to give the desired hapten III (0.82 g, 80% yield) as pale-yellow flakes, mp 98–102°C, which showed one spot on TLC: R_f 0.15 (benzene–methanol–diethylamine) (100:5:5) (II, R_f 0.25); IR: 1025 cm^{-1} (S → O); ^{1}H-NMR ($CDCl_3$): δ 2.1 (t, 2, J = 6.0 Hz, propyl central CH_2), 2.5 (m, 14, piperazine methylene, CH_2-piperazine, and CH_2CH_2—COOH), 4.2 (t, 2, J = 6.0 Hz, CH_2-phenothiazine), 7.8 (m, 7, ArH), and 10.0 ppm (s, 1, COOH); MS (ammonia CI): quasi-molecular ion $(M + H)^+$ at m/z 482 for III and m/z 496 for the methyl ester of III.

Anal.—Calc. for $C_{23}H_{26}F_3N_3O_3S$: C, 57.38; H, 5.41; N, 8.73. Found: C, 58.00; H, 5.57; N, 8.66.

The hapten III (0.130 g) was coupled to bovine serum albumin[8] (0.19 g) in dioxane by using a modified mixed-anhydride method (12, 13). This was followed by dialysis first against bicarbonate buffer (0.1 M; pH 8), then against acetate buffer (0.1 M; pH 5.2), and finally against distilled water. During the dialysis against acetate buffer, there was some precipitation, which disappeared when dialyzed again with bicarbonate buffer. After lyophilization, the immunogen IV was obtained as a white crystalline solid (0.27 g). A blank was prepared by subjecting the bovine serum albumin to the coupling conditions, but without the hapten. The number of hapten residues coupled per mole of bovine serum albumin conjugate was determined by the UV[9] method (12, 13) to be 60.

Synthsis of the Sulfoxide Metabolite of [^{3}H]Trifluoperazine—To a solution of [^{3}H]trifluoperazine[3] in ethanol (200 μL, 1 mCi/mL) was added 20 μL of concentrated HNO_3 (11 M). The reaction mixture was left in the dark at room temperature for 3 h and subsequently diluted with water (5 mL), basified with 0.2 mL of saturated sodium carbonate, and extracted with benzene (5 × 3 mL). The benzene was evaporated under a stream of nitrogen at 65°C[10]. The residue, which was reconstituted with 0.2 mL of methanol, was injected into the HPLC system[11], which was operated at ambient temperature with a flow rate of 3 mL/min. The eluted peak corresponding to the sulfoxide metabolite (2.5–3 min) was collected.

To determine the specific activity of the tracer, aliquots of the collected fraction were injected into the HPLC system. The amount of tritiated sulfoxide metabolite was determined by comparing the peak height of the sulfoxide metabolite of [^{3}H]trifluoperazine to that obtained from injections of known amounts of unlabeled metabolite. The tritiated sulfoxide metabolite peak was collected and counted by a liquid scintillation technique. The specific activity of the tritiated sulfoxide metabolite was calculated to be ~12.2 Ci/mmol. The chemical yield was found to be 50.5% by comparing the ratio of the radioactivity of the tritiated sulfoxide product with that of the [^{3}H]trifluoperazine used in the reaction.

The purity of tracer, when required for RIA, was checked by TLC: R_f 0.2 (benzene–methanol–diethylamine; 100:5:5). The purity was always >95%. The working solution of the tritiated sulfoxide metabolite was prepared on the day of assay by diluting the required quantity of the metabolite, freshly collected from the HPLC system, with 0.01 M HCl such that the diluted solution contained 2000–2500 cpm/μL.

Pilot synthesis experiments with unlabeled trifluoperazine using the oxidation procedure and isolation techniques described above gave the sulfoxide metabolite of trifluoperazine as white crystals, uncorrected mp 139–140°C [lit. (9) mp 140–141°C], which gave satisfactory C, H, and N analytical data.

Immunization—Four New Zealand White rabbits[12] (age, 4 months) were each given an intradermal injection of 1 mg of the immunogen emulsified with 0.5 mL of Freund's complete adjuvant–0.5 mL of isotonic saline. Thereafter, rabbits were reimmunized by intradermal injection at 2-week intervals with the same amount of immunogen emulsified with Freund's incomplete adjuvant. After five intradermal injections, the sera of three rabbits showed binding activity to the sulfoxide metabolite of [^{3}H]trifluoperazine. After administration of four intravenous booster doses of 1 mg of the immunogen in 0.25 mL of isotonic saline, which were given *via* the ear vein at 2-week intervals, the three rabbits produced antisera of adequate titer (1:1300 w/v). These rabbits were then sacrificed, and the antisera of each of the rabbits was lyophilized and stored at −20°C. The lyophilized antiserum of one of the rabbits was used for the RIA of trifluoperazine sulfoxide.

Direct Radioimmunoassay Procedure—An aqueous solution of the sulfoxide metabolite of trifluoperazine was prepared by dissolving the sulfoxide in 0.01 M HCl whenever required. Subsequently, standard solutions containing 0.3, 0.5, 1, 2, 3, 5, 7.5, and 10 ng of the sulfoxide/mL were prepared in pooled plasma[13]. The working antiserum solution was prepared by dissolving the lyophilized antiserum in phosphate buffer (0.2 M, pH 7.2). Of this solution, 100 μL was used in each assay tube.

To each polystyrene tube (12 × 75 mm), containing a 200-μL plasma

[1] A gift from Smith, Kline and French Laboratories, Philadelphia, Pa.
[2] Rhône-Poulenc Pharma Inc., Montreal, Quebec, Canada.
[3] Nuclear Research Centre-Negev, Beer-Sheva, Israel.
[4] Ready Solve-MP; Beckman Instruments Inc., Fullerton, Calif.
[5] LKB RackBeta, model 415; Fisher Scientific Co., Toronto, Ontario, Canada.
[6] Terochem Laboratories Ltd., Toronto, Ontario, Canada.
[7] Melting points were determined with a Gallenkamp melting point apparatus and are uncorrected. TLC was performed with Eastman chromatogram sheets, type 13254 (silica gel with fluorescent indicator); spots were observed under shortwave UV light. ^{1}H-NMR spectra were determined in $CDCl_3$ or Me_2SO-d_6 on a Varian T-60 instrument with tetramethylsilane as the internal reference. Low-resolution electron-impact (EI) and chemical-ionization (CI) mass spectra were recorded on a VG Micromass MM16F instrument at 70 eV equipped with a VG 2025 data system. IR spectra were recorded on a Perkin-Elmer 297 instrument (KBr pellets). All EI, CI, ^{1}H-NMR, and IR spectra of synthesized compounds were consistent with assigned structures. Microanalyses for samples dried over phosphorus pentoxide at 60°C under reduced pressure were performed by Mr. R. E. Teed, Department of Chemistry, University of Saskatchewan. All reagents were purchased from Aldrich Chemical Co., Montreal, Quebec, Canada.
[8] Sigma Chemical Co., St. Louis, Mo.
[9] Pye Unicam SP 1700 spectrophotometer.
[10] Thermolyne Dri-Bath; Fisher Scientific Co., Edmonton, Alberta, Canada.
[11] Solvent delivery system (M45; Waters Associates, Milford, Mass.), a valve loop injector fitted with a 1-mL loop (model 7120; Rheodyne, Berkley, Calif.), a radial compression separation system (Z module; Waters Associates), mobile phase composed of 10% sodium acetate buffer (0.1 M; pH 6) in methanol, and a variable-wavelength UV detector (Lambda Max model 480; Waters Associates) fixed at 254 nm.
[12] Mr. Avaldermeer, Sherwood Park, Alberta, Canada.
[13] Canadian Red Cross.

Table IV—Pharmacokinetic Parameters of Trifluoperazine and Its Sulfoxide Metabolite [a]

Subject	Age, years	Weight, kg	T_{max}, h		C_{max}, ng/mL		AUC_{0-24}, ng·h/mL [b]		AUC_{0-24} Metabolite/ AUC_{0-24} Drug, %	$t_{1/2}$, h	
			Metabolite	Drug	Metabolite	Drug	Metabolite	Drug		Metabolite [c]	Drug [d]
1	40	67	4.5	4.5	2.9	1.9	36.3	17.3	210	7.2	10.8
2	40	80	6	3	3.3	3.2	33.9	29.4	115	5.4	7.8
3	22	88	4.5	4.5	3.1	2.5	34.9	22.0	159	6.4	2.5
4	32	74	3	3	2.5	1.1	36.8	9.1	404	6.2	15.2
5	21	94	4.5	3	4.5	1.0	56.9	8.0	711	3.7	8.1
Mean ± *SD*	31 ± 9.2	80.6 ± 10.8	4.5 ± 1.1	3.6 ± 0.8	3.3 ± 0.7	1.9 ± 0.9	39.8 ± 9.6	17.2 ± 8.9	320 ± 245	5.8 ± 1.3	8.9 ± 4.6

[a] Pharmacokinetic parameters of trifluoperazine and its sulfoxide metabolite in the plasma of five healthy volunteers after a 5-mg oral dose of trifluoperazine. [b] AUC_{0-24} was calculated by the trapezoidal rule. [c] Plasma half-life was calculated by the equation $t_{1/2} = 0.693\ k_{el}$, where k_{el} was estimated by using the plasma concentrations between 6 and 24 h. In the case of volunteer 4, the 24-h plasma concentration was estimated, since there was found to be <0.3 ng/mL of the sulfide metabolite present. [d] Plasma half-life was calculated by the equation $t_{1/2} = 0.693/k_{el}$, where k_{el} was estimated by the plasma concentration between 4.5 and 24 h for subjects 1 and 2 and between 4.5 and 12 h for subjects 3–5.

sample (standard or from a volunteer), was added 200 μL of phosphate buffer (0.2 M; pH 7.2) and 5 μL of the sulfoxide metabolite of [^{3}H]trifluoperazine solution (10,000–12,500 cpm). The contents of the tube were mixed[14] for 10 s, and 100 μL of antiserum solution (1:1300 w/v) was added. All of the additions were carried out in an ice bath. The solution was mixed[14] well for 10 s and incubated at 37°C for 80 min. The tubes were then cooled to 4°C in an ice bath, and cold dextran-coated charcoal suspension (1 mL) was added. Each tube was again mixed[14] for 10 s and subsequently incubated for 10 min at 4°C. The samples were then centrifuged (1720×*g* for 10 min at 4°C). The supernatant solution was decanted by a standardized procedure into a scintillation vial containing 10 mL of scintillation cocktail, mixed well, and counted for 5 min. Each plasma sample, standard or unknown, was assayed in triplicate.

The calibration curve was constructed by plotting the logit values of B/B_0 on the *y*-axis and the log values of the sulfoxide metabolite concentrations on the *x*-axis, where B and B_0 are the antibody-bound radioactivity in the presence and absence of the sulfoxide metabolite, respectively. Logit B/B_0 is defined as:

$$\text{Logit } B/B_0 = \text{Log}_e \frac{B/B_0}{1 - B/B_0}$$

Extraction Radioimmunoassay Procedure—Each 1-mL plasma sample (standard or from a volunteer) was basified (pH 12) with saturated Na_2CO_3 (1 mL) and extracted with benzene (2 × 3 mL). The resultant extract was evaporated to dryness under a stream of nitrogen at 65°C, and the residue was reconstituted with 1 mL of phosphate buffer (0.2 M; pH 7.2). Subsequently, the RIA procedure described above was followed, in which 200 μL of 7% aqueous bovine serum albumin solution and 200 μL of reconstituted extract were used in the place of plasma and phosphate buffer, respectively.

The efficiency of the benzene extraction of the sulfoxide metabolite of trifluoperazine was checked by repeating the extraction procedure with spiked plasma samples containing known amounts of the tritiated sulfoxide. Of the total radioactivity added, 98% was found to be extracted from samples containing 0.3–10 ng/mL of the sulfoxide metabolite of trifluoperazine.

Plasma Samples—A 5-mg dose of trifluoperazine[15] was given orally with 50 mL of water to each of five healthy male volunteers, who fasted overnight[16]. Blood samples were withdrawn by venipuncture using evacuated heparinized tubes[17], and contact between blood and the rubber stopper was carefully avoided, as spurious results have been found in the RIA determination of trifluoperazine when blood has been allowed to touch the rubber stopper of these evacuated tubes (14). Blood samples were taken at 0 (predose), 0.5, 1, 1.5, 2, 3, 4.5, 6, 8, 12, and 24 h postdose. The plasma was immediately separated and stored at −20°C until analysis by the procedures described above for the sulfoxide metabolite and by the previously reported RIA method for trifluoperazine (6).

RESULTS

Sensitivity and Specificity of the Radioimmunoassay for the Sulfoxide Metabolite of Trifluoperazine—The amount bound at a zero concentration of the sulfoxide metabolite was determined at 30, 60, 80, 120, and 180 min and at temperatures of 4°C, 22°C (room temperature), and 37°C for the first incubation step in the assay procedure. From the results of these experiments, the optimal conditions for the assay were found to be an incubation time of 80 min at 37°C, where the B_0 was ~30%.

When 200-μL plasma samples are used, as low as 60 pg of the sulfoxide can be measured by the RIA. Under the assay conditions described above, the standard curve was linear from 0.3 to 10 ng/mL; a typical calibration curve can be defined by: logit $y = -2.27 \cdot \log_{10} x + 1.496$ ($r^2 = 0.999$). This range was adequate for the determination of all but one of the unknown concentrations of the sulfoxide metabolite in the plasma samples obtained after administration of a single oral dose of 5 mg of trifluoperazine. These concentrations were determined by running a calibration curve with each set of unknown samples. Concentrations of the sulfoxide metabolite of <0.3 ng/mL, reported for one plasma sample in the present study, were estimated.

The specificity of the antiserum was assessed by the criteria of Abraham (15). The antiserum did not cross-react markedly with trifluoperazine or the available, supposed, major metabolites of trifluoperazine; however, the cross-reactivity of the *N*-demethyl sulfoxide metabolite of trifluoperazine as well as the sulfoxide metabolites of other piperazine-type phenothiazines is understandable (Table I). Interestingly, the sulfoxide of the aliphatic phenothiazine, chlorpromazine, did not cross-react.

Also, to check cross-reactivities, plasma samples containing the sulfoxide metabolite of trifluoperazine over the standard curve range were additionally spiked with trifluoperazine at five times the sulfoxide concentrations, and the sulfoxide concentrations were subsequently determined by the direct RIA procedure. These experiments were repeated an additional four times, and the values obtained were compared with those for the sulfoxide metabolite standard curve samples prepared at the same time in the usual manner. There were no significant differences ($p > 0.05$) in the values obtained for the sulfoxide metabolite among samples of the same sulfoxide concentrations with or without trifluoperazine. Similar results were obtained when the 7-hydroxy, *N*-oxide, and *N*-demethyl metabolites were each separately added at five times the sulfoxide concentrations to plasma samples containing the sulfoxide over the concentration range of the standard curve (Table II).

Intra- and Interassay Variations—Both intra- and interassay variations were determined with plasma standards prepared in accordance with the described procedure. Intra-assay variations were determined from assay readings obtained from a single day, whereas interassay variations were calculated from the assay data obtained on four separate days of analysis (Table III). The *CV* was <4% for both intra- and interassay determinations at all the sulfoxide concentrations studied.

Plasma Concentrations of the Sulfoxide Metabolite of Trifluoperazine and Trifluoperazine—The plasma concentration–time profiles (Fig. 1) obtained by direct RIA after administration of a single 5-mg oral dose of trifluoperazine to five healthy volunteers showed considerable intersubject variations in the plasma concentration of the sulfoxide metabolite and trifluoperazine. The sulfoxide metabolite was present in detectable concentrations at all sampling times. However, in three volunteers (subjects 3–5), no trifluoperazine was detected in the 24-h plasma sample.

The areas under the plasma concentration–time curve from 0 to 24 h (AUC_{0-24}) were calculated by the trapezoidal rule and ranged from 33.9 to 56.9 ng·h/mL for the sulfoxide metabolite and 8.0 to 29.4 ng·h/mL for trifluoperazine. The values of $AUC_{0\ 24}$ for the sulfoxide metabolite were, on the average, 320% of those of trifluoperazine (range, 115–710%). The time required to reach the peak concentrations (t_{max}) in plasma were similar for the sulfoxide metabolite and trifluoperazine and ranged from 3 to 4.5 h, except in one volunteer (subject 2), for whom the t_{max} for the sulfoxide was 6 h. The peak concentrations (C_{max}) ranged from 2.5 to 4.5 ng/mL for the sulfoxide metabolite and from 1.0 to 3.2 ng/mL for trifluoperazine (Table IV).

After reaching these C_{max} values, plasma concentrations declined rapidly in all subjects, so that for the sulfoxide metabolite, the elimination half-life ($t_{1/2}$) from 6 to 24 h was calculated to range from 3.7 to 7.2 h in the five subjects, whereas for trifluoperazine the $t_{1/2}$ was in the range of 2.5–15.2 h. In the latter case, $t_{1/2}$ (4.5–24 h) was calculated for the only two subjects, in

[14] Vortex Genie; Fisher Scientific Co.
[15] Stelazine; Smith, Kline and French Laboratories.
[16] Protocol was approved by the local Ethics Committee on Human Experimentation.
[17] Vacutainer; Becton and Dickinson, Co., Mississauga, Ontario, Canada.

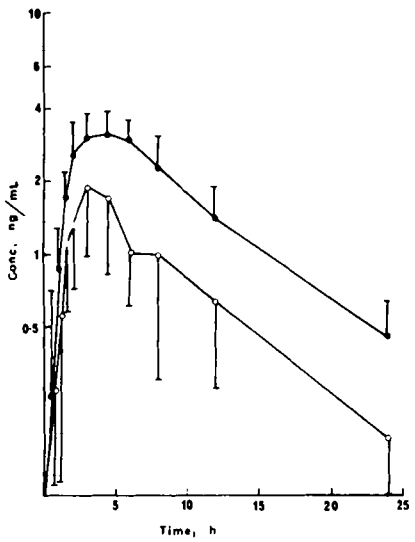

Figure 1—*Mean plasma trifluoperazine (○) and sulfoxide metabolite (●) concentrations in five healthy volunteers after single 5-mg oral doses of trifluoperazine dihydrochloride. Each point represents mean ± SD.*

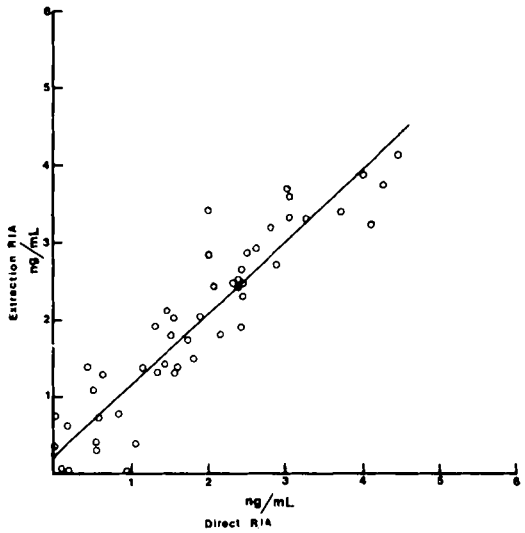

Figure 2—*Comparison of plasma concentrations of the sulfoxide metabolite of trifluoperazine determined for five volunteers after ingestion of 5 mg of trifluoperazine and analyzed by the extraction RIA and the direct RIA procedures (n = 50, r^2 = 0.92, slope = 0.89).*

whom the trifluoperazine plasma concentrations at 24 h could be determined by RIA, whereas for the other three volunteers, $t_{1/2}$ was calculated from the concentration time points at 4.5–12 h. Closer inspection of the data indicated that in the case of one volunteer (subject 2), plasma concentrations of trifluoperazine and the sulfoxide metabolite declined biexponentially after the C_{max} was reached. For this subject, the ($t_{1/2,\alpha}$) and ($t_{1/2,\beta}$) values were calculated as 1.9 and 12.8 h, respectively, for the sulfoxide, whereas the analogous values for trifluoperazine were 1.4 and 16.4 h. All plasma half-lives were estimated from the raw data using the equation $t_{1/2} = 0.693/k_{el}$, where k_{el} is the calculated rate constant of elimination.

The plasma concentrations of the sulfoxide metabolite of trifluoperazine for all plasma samples obtained from the five volunteers were also determined by the extraction RIA procedure. The values obtained were plotted *versus* those obtained by the direct RIA (Fig. 2). The slope of the regression line for this comparison had a value of 0.89, with r^2 = 0.92. The 95% confidence bounds (±0.179) of the slope of this regression line were calculated, and thus, the slope is not significantly different statistically from 1.0. Also, when both techniques were performed on three separate occasions for one of the volunteers (subject 5) and the mean of the extraction technique values were plotted *versus* the mean of the direct RIA technique, the correlation coefficient was determined to be 0.98, and the slope of the regression line was 0.80. These results indicate that the two methods compare favorably.

DISCUSSION

The previously reported trifluoperazine hapten (II) was the starting material used for the synthesis of the desired sulfoxide hapten (III). This starting material (II) was prepared as previously described (11) or by an equally successful alternate routine (Scheme I), in which the *N*-demethylmetabolite of trifluoperazine (Ia) was treated successively with methyl acrylate and base. Subsequent nitric acid oxidation of II gave the desired hapten (III) in good yield, although the relatively high water solubility of this compound necessitated saturation of the reaction mixture with sodium chloride so that organic solvent extraction of the product was feasible. A similar nitric acid treatment procedure was adopted to obtain the tracer required for the RIA of the sulfoxide metabolite of trifluoperazine in which the commercially available ethanolic solution of [^{3}H]trifluoperazine was directly oxidized: The tritiated sulfoxide product was identified by its chromatographic properties (TLC and HPLC), as well as by indirect pilot experiments in which unlabeled trifluoperazine was treated by the same nitric acid procedure and the product was examined by qualitative spectral analysis (CI-MS, EI-MS, IR, ^{1}H-NMR), as well as by elemental analysis.

The development of the present RIA was based on the most successful of the approaches taken in order to develop an RIA for trifluoperazine, in which the drug molecule was linked to bovine serum albumin *via* a two-carbon bridge attached through the N-10 side chain of the trifluoperazine structure (11). The antisera raised to such an immunogen should be specific to the ring portion of the drug molecule, and indeed, the sulfoxide antisera reported here did not cross-react with trifluoperazine, whereas there was significant cross-reactivity with the sulfoxide metabolites of the other available marketed piperazine-type phenothiazines, *i.e.*, fluphenazine, perphenazine, and prochlorperazine. Thus the antisera can be used for the quantitative RIA analysis of the sulfoxide metabolites in plasma. Antibody recognition was not dramatically affected when the 2-trifluoromethyl ring substituent of the sulfoxide metabolite of trifluoperazine was altered to a 2-chloro substituent, as with the sulfoxide metabolite of perphenazine, yet the 2-thioether, a thioridazine ring sulfoxide which has a piperidine side chain, was not recognized by the antibody. Also, regarding other ring-substituted compounds, there was no measured cross-reactivity with the 7-hydroxy metabolite of trifluoperazine, whereas the sulfone metabolite of trifluoperazine demonstrated low binding activity.

This is the first report of a suitable analytical method for the quantitation of the sulfoxide metabolite of trifluoperazine in plasma. This RIA procedure can measure 60 pg of the sulfoxide metabolite in a 200-μL plasma sample, thus obviating the need for large blood samples. Also, similar results were obtained for the assay irrespective of whether the procedure was carried out directly on plasma or on plasma extracts. Thus, a plot of the B/B_0 values obtained in the direct RIA for various plasma standards, covering the range 0.3–10 ng of the sulfoxide per mL, *versus* those obtained in the extraction RIA procedure for the same plasma standards, gave an r^2 value of 0.998.

Regarding the specificity of the antiserum (Tables I and II), there were no significant changes in binding when plasma standards of the sulfoxide metabolite over the standard curve concentration range were spiked with trifluoperazine at concentrations five times those of the sulfoxide. Therefore, it is unlikely that trifluoperazine will interfere with sulfoxide plasma concentration determinations in samples from healthy volunteers or patients treated with trifluoperazine. Also, the available suspected major metabolites of trifluoperazine in humans (the 7-hydroxy, *N*-oxide, and *N*-demethyl compounds) failed to demonstrate cross-reactivity either by the criteria of Abraham (15) or when added to spiked samples of the sulfoxide metabolite at five times the concentration of the sulfoxide. However, as expected, the *N*-demethyl sulfoxide metabolite cross-reacts appreciably with the antiserum. Whether or not the concentrations of the *N*-demethyl sulfoxide metabolite in plasma samples from patients or healthy volunteers are high enough to interfere in the determination of the sulfoxide concentrations awaits study of the levels of this metabolite in humans after single-dose and chronic administration of trifluoperazine. Additional proof for the specificity of the RIA procedure for the sulfoxide metabolite was obtained by comparison of the data for extraction RIA with that for direct RIA determined for the plasma samples from the volunteers dosed with a single 5-mg oral dose of trifluoperazine. Similar results were obtained irrespective of whether the procedure was carried out directly on plasma or on plasma extracts, indicating that physiological material and metabolites nonextractable in benzene do not dramatically interfere in the sulfoxide determinations.

Plasma concentration–time profiles for trifluoperazine after administration of low, single, oral doses of trifluoperazine, in which trifluoperazine plasma concentrations were determined by RIA, have been previously reported (16). The mean values for this previous study with 24 healthy male volunteers, in which the t_{max}, C_{max}, $AUC_{0\text{-}24}$, and $t_{1/2}$ values for one of the tested 5-mg trifluoperazine formulations were 4.0 ± 1 h, 1.9 ± 0.9 ng/mL, 19.3 ± 11

ng·h/mL, and 9.3 ± 7 h, respectively, are in agreement with the values reported here of 3.6 ± 0.8 h, 1.9 ± 0.9 ng/mL, 17.2 ± 9 ng·h/mL, and 8.9 ± 5 h, respectively, for the five volunteers given a single 5-mg oral dose of trifluoperazine. On the other hand, pharmacokinetic parameters in humans for the sulfoxide metabolite of trifluoperazine have not been previously reported. The mean sulfoxide concentrations were of the same order as the mean trifluoperazine concentrations at the first (0.5 h) time point and thereafter were greater (Fig. 1). The presence of significant amounts of the sulfoxide (98% of trifluoperazine) at the 0.5-h sampling point and the fact that the plasma sulfoxide concentrations peaked at about the same time as the plasma trifluoperazine concentrations indicates that a significant proportion of trifluoperazine is metabolized to the sulfoxide metabolite during presystemic absorption. Also, in all volunteers the percent AUC_{0-24} sulfoxide-AUC_{0-24} trifluoperazine ratio was >100%. The 115–710% range for this value in the five volunteers is indicative of large interindividual variation resulting from presystemic metabolism of trifluoperazine by routes such as sulfoxidation.

For four of the five subjects, the terminal elimination half-life of trifluoperazine was longer than that of the more polar sulfoxide metabolite, which suggests a generally faster elimination of the sulfoxide. A similar phenomenon has been reported on comparison of the sulfoxide metabolite of chlorpromazine and chlorpromazine terminal elimination half-lives after administration of low, single, oral doses of chlorpromazine to healthy male volunteers (17). However, pharmacokinetic data obtained from the RIA measurement of plasma concentrations should be viewed with suspicion. Indeed, the RIA antisera used here for trifluoperazine plasma concentration determinations cross-reacts with the *N*-demethyl and 7-hydroxy metabolites of trifluoperazine to the extent of 26 and 24%, respectively (6). When these antisera were used to determine plasma concentrations in an oral single-dose bioequivalency study of two trifluoperazine tablet formulations in five healthy volunteers, a longer mean elimination half-life (11.5 ± 3.7 compared with 8.3 ± 2.4 h for one of the formulations) was found than when the plasma concentrations were determined for the same plasma samples by a specific GC–MS method (18). The RIA method for the sulfoxide metabolite needs to be compared with a sensitive and specific chemical method for the determination of the sulfoxide in plasma to validate the pharmacokinetic data reported here. Due to such reasons as the demonstrated heat sensitivity of the sulfoxide metabolite of trifluoperazine (19) and the low concentrations encountered after administration of this highly potent antipsychotic agent, such development will not be easy. In the meantime, due to the specificity demonstrated in this report, the RIA for the sulfoxide metabolite can be investigated as to its suitability to compare measured sulfoxide concentrations in responding and nonresponding patients under chronic treatment with trifluoperazine. The resultant data will assist in establishing the relationship, if any, between plasma concentrations of the sulfoxide metabolites of trifluoperazine, therapeutic response, and/or adverse drug reactions.

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