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Chlorpromazine Metabolism VIII: Blood Levels of Chlorpromazine and Its Sulfoxide in Schizophrenic Patients

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Abstract □ A procedure was standardized for extracting chlorpromazine and its sulfoxide from the blood and for applying a recently developed fluorometric assay method to determine blood levels of these two compounds in schizophrenic patients receiving chlorpromazine therapy. The described methodology opens avenues for performing bioavailability and generic equivalence studies in humans.

Keyphrases □ Chlorpromazine and its sulfoxide—extraction from whole blood, fluorometric analysis □ Tranquilizers—chlorpromazine and its sulfoxide, extraction from whole blood, fluorometric analysis □ Fluorometry—analysis, chlorpromazine and its sulfoxide after extraction from whole blood

An approach to assaying chlorpromazine and its sulfoxide in relatively low concentrations was described recently (1–4). It utilizes quaternization of the tertiary amines with 9-bromomethylacridine and a photolytic process to generate fluorescent acridine derivatives in a stoichiometric fashion. The chief advantages of this methodology include a relatively high sensitivity, adequate for determining the clinically accrued blood levels; a high degree of precision, not present in any other method available for assaying chlorpromazine on a routine basis; and ease of analysis of a large number of samples simultaneously.

This paper describes the biological and clinical applications of the fluorometric methodology, preliminary to detailed clinical and pharmacokinetic studies on chlorpromazine currently in progress.

EXPERIMENTAL

Materials and Equipment—The materials and equipment were described previously (4). Isoamyl alcohol, 1-propanol, ether, toluene, *n*-heptane[1], and various chlorpromazine metabolites[2] were also used.

Assay Procedure—The optimum assay procedure, developed and standardized previously (4), was used except that chlorpromazine and its sulfoxide had to be isolated from the blood by an appropriate extraction procedure prior to application of the assay method. Therefore, the extraction procedure was standardized as to solvent, pH, and shake-time for optimum extraction.

Extraction Procedure—The use of sonicated whole blood was preferred over plasma, since chlorpromazine (5) as well as its metabolites (6, 7) is localized in the erythrocytes. The sample size was limited to 3 ml as in other assays (7), since volumes of 5 ml or less are clinically reasonable. However, larger volumes may be used if necessary when assaying chlorpromazine levels below 20 ng/ml.

Extraction Solvent—To seven sets of three tubes, each containing 3.16 nmoles of chlorpromazine in 3 ml of sonicated blood adjusted to pH 13, was added 10 ml each of various organic solvents in a 2:1 organic to aqueous volume ratio. The tubes were shaken for a constant time and centrifuged. A 9-ml aliquot of the organic solvent was removed and processed through the remainder of the procedure.

Extraction pH—A series of extraction tubes each contained a 3-ml sonicated blood sample with 3.16 nmoles of chlorpromazine and its sulfoxide. The samples were adjusted to various pH values between 9 and 14, with a set of three to five tubes per pH value. The samples were extracted for a constant time with *n*-hexane containing 1.5% isoamyl alcohol. The extracts were evaporated to dryness under nitrogen and at room temperature and processed through the assay procedure to determine the optimum pH for extraction.

Duration of Extraction—Sets of extraction tubes, with each tube containing fixed known quantities of chlorpromazine and its sulfoxide in 3-ml blood samples at pH 13 and 10 ml of an extraction solvent, were shaken horizontally in a mechanical shaker at 150 cpm for various lengths of time. The tubes were centrifuged,

[1] All nanograde or analytical reagent grade, Mallinckrodt Chemical Works, St. Louis, Mo.
[2] Obtained from the Psychopharmacology Research Branch, National Institute of Mental Health.

and appropriate aliquots of the organic phase were evaporated and processed by the assay procedure to determine the optimum shake-time for extraction.

Evaporation of Extract—After centrifugation for 10 min at 2250 rpm following the extraction phase, 9-ml aliquots of the organic layer from each tube were transferred to 30-ml Pyrex tubes and evaporated under nitrogen at different temperatures to approximately 0.5 ml. The concentrated extract was quantitatively transferred to a 1.5-ml stoppered polyethylene tube with the help of sequential rinses of 1.0, 0.5, and 0.5 ml of methanol. The solvent was evaporated to dryness and subjected to the assay procedure.

Addition and Recovery—Known quantities of chlorpromazine and its sulfoxide were added to 3-ml sonicated blood samples and processed through optimum extraction and assay procedures. The same quantities of the phenothiazines were also added directly to the quaternization reaction tubes and carried through the assay procedure.

Standard curves for the two compounds were obtained by adding varying amounts of each compound to naive human blood and then assaying the blood to determine the relative fluorescence corresponding to the various concentrations of the compounds.

Clinical Data—The standardized extraction and assay procedure is currently being used to analyze blood levels of chronic schizophrenic patients who received 200 or 600 mg/m^2/day po of chlorpromazine in four equally divided doses. A kinetic study was carried out on these patients at the beginning of therapy (Week 0) and at Weeks 4 and 12. For 3 days prior to and after each kinetic study, the patients were maintained on a standard liquid diet of uniform composition and calorie value.

On the day of the kinetic study, 120 mg/m^2 po of chlorpromazine hydrochloride in a syrup formulation was administered before breakfast. No food was given until 2 hr after the oral dose. Also, no medication was given following that single kinetic study dose until the 3rd day when the chlorpromazine four times a day schedule was resumed. No other drug was given to the patients. The blood samples were drawn by aseptic venipuncture before and at various time intervals after the kinetic study dose and kept frozen until assayed.

RESULTS AND DISCUSSION

The extraction efficiency of chlorpromazine was enhanced when the blood samples were extracted at pH 12–14 rather than at pH 9–11. A pH of 13 and above was chosen for routine extractions, because hydroxylated tertiary amine metabolites were not extracted into the organic solvent to any meaningful extent (less than 5%) at that pH. The optimum shake-time for extraction was 30 min.

Of the various organic solvents (Table I) evaluated for single-extraction efficiency, toluene, *n*-heptane, and *n*-hexane, all containing 1.5% isoamyl alcohol, were equally effective in extracting chlorpromazine from the blood at pH 13. Being a relatively lower boiling solvent, *n*-hexane was selected for routine extractions. Although ether was equally good, the quantitative transfers of this solvent were generally inaccurate due to its relatively high vapor pressure.

The evaporation of the organic extract was always carried out under nitrogen, since it is known that the phenothiazines are readily oxidizable. Of the various temperatures tested for suitability for evaporation of the extract, room temperature appeared to yield most consistent results.

Optimum Extraction Procedure—A 3-ml whole blood sample, containing chlorpromazine and its sulfoxide to be assayed, was sonicated in an extraction tube and adjusted to pH 13.0–13.5 with 2 *N* NaOH. Ten milliliters of *n*-hexane containing 1.5% isoamyl alcohol was added. The tube was stoppered, shaken horizontally on a mechanical shaker at 150 cpm for 30 min, and centrifuged. A 9-ml aliquot of the organic phase was transferred to another tube for evaporation to 0.5 ml at room temperature and under nitrogen.

The concentrate was quantitatively transferred to a 2.5-ml polyethylene reaction tube with the help of three sequential rinses of 1.0, 0.5, and 0.5 ml of methanol. The combined transfer rinses were evaporated to dryness at room temperature and under nitrogen. The residue was then treated with the quaternizing reagent and processed by the optimum assay procedure.

Addition and Recovery—Based on single extractions under optimum conditions of pH, shake-time, and solvent, the percent recoveries of various amounts of chlorpromazine added to blood ranged from 65 to 74% (Table II). The precision of replicate extractions was well within ±5%.

The data obtained by adding known amounts of chlorpromazine and its sulfoxide to 3-ml samples of blood and assaying by the combined extraction and assay procedure yielded linear standard curves for the two compounds over a concentration range of 20 ng–1 μg/ml.

Precision and Blanks—The precision of over 200 replicate (n = 2–5) sets of analyses, carried out over several months and expressed in terms of coefficient of variation, was ≤5% for nearly 80% of the total sets. Replicate analyses yielding a coefficient of variation larger than 10% are subject to a repeat assay in this laboratory. Considering the number and nature of various steps involved in the assay, the observed precision appeared fairly good.

The relative fluorescence values of blood blanks, inherently due to contributions of the blood extractables (drug unrelated) and the TLC plates, are shown in Table III. On the whole, the replicates of blank values within an experiment varied to a far less extent (<5%) than did the values between the experiments on different days. This variation was related to the variation in the source of blood. In routine analyses of chlorpromazine and its sulfoxide in blood, the corresponding fluorescence values of the blanks were subtracted from those of the unknown samples assayed, prior to computation of the unknown concentrations, with the help of standard curves.

Interference by Metabolites—The extraction procedure was designed to exclude all the hydroxylated metabolites of chlorpromazine. The primary and secondary amine metabolites, although extracted from the blood along with the drug and its sulfoxide, either do not react with the acridine reagent or their reaction products move to the solvent front along with the other relatively nonpolar reagent and reaction by-products when subjected to TLC with the acetonitrile–water system.

Theoretically, other nonhydroxylated metabolites of chlorpromazine, *e.g.*, 7-methoxychlorpromazine and its sulfoxide, should also be extractable if present in the blood of patients receiving chlorpromazine therapy. However, preliminary experiments indicated that this may not be a major problem. For example, when a 10-ml blood sample (at the peak absorption time) of a patient receiving a 1600-mg chronic daily dose of chlorpromazine was ex-

Table I—Relative Extractability of Chlorpromazine from Blood at pH 13 into Various Organic Solvents following Single Extraction for 30 min at 150 Shakes/min

Solvent	Maximum Extraction, %
n-Hexane	28
Benzene	38
15% 1-Propanol in hexane	43
3% Isoamyl alcohol in hexane	66
Ether	100
1.5% Isoamyl alcohol in *n*-heptane	100
1.5% Isoamyl alcohol in toluene	100
1.5% Isoamyl alcohol in *n*-hexane	100

Table II—Addition of Chlorpromazine to, and Its Recovery from, Whole Human Blood

Amount Added, ng/ml	Amount Recovered, ng/ml	Recovery, %
40	26 ± 1.6[a]	64.0 ± 2.9
60	39 ± 0.8	64.0 ± 1.4
100	71 ± 2.9	71.0 ± 2.6
400	279 ± 17.3	70.0 ± 3.7

[a] Mean ± *SD*; n = 2–4.

Table III—Relative Fluorescence Values of Various Blanks

Blank	Relative Fluorescence
Plate	70–90
Reaction (including plate blank)	100–120
Blood (including reaction blank)	120–220

Table IV—Blind Analyses of Spiked Blood Samples Containing Chlorpromazine and Its Sulfoxide

Chlorpromazine Concentration, ng/ml		Chlorpromazine Sulfoxide, ng/ml	
Added	Found	Added	Found
30.7[a]	29.0	30.3[a]	32.3
	27.5		30.6
	28.5		30.2
	25.0		33.1
	29.5		35.6
	$\bar{x}$[b] = 28.0 ± 1.88		$\bar{x}$ = 32.0 ± 2.20
139.0[c]	153.0	128.0[c]	115.0
	142.8		116.3
	140.0		112.0
	$\bar{x}$ = 145.0 ± 6.84		$\bar{x}$ = 114.0 ± 2.20
278.0[c]	278.0	256.0[c]	234.5
	251.5		224.3
	283.8		284.3
	$\bar{x}$ = 271.0 ± 17.20		$\bar{x}$ = 231.0 ± 6.90
256.0[a]	298.0	306.0[a]	313.0
	305.0		330.0
	311.0		333.0
	320.0		335.0
	295.0		325.0
	$\bar{x}$ = 306.0 ± 10.10		$\bar{x}$ = 327.0 ± 8.80

[a] Lyophilized plasma samples prepared by Dr. W. J. Turner, Central Islip State Hospital, Long Island, N.Y. [b] Mean ± *SD*. [c] Prepared at Central State Griffin Memorial Hospital.

tracted and the extract was subjected to TLC with methanol–ethyl acetate–acetic acid–water (17:56:17:10) as the developing solvent, 7-methoxychlorpromazine and its sulfoxide were nondetectable (authentic compounds used as reference). However, traces of promazine, its sulfoxide, and 7-methoxypromazine were detected. On elution, evaporation under nitrogen to dryness, and subjection to the described assay procedure, these promazine compounds at the peak absorption time together constituted nearly 30% of the steady-state blood level (800 ng/ml) of the patient measured in terms of chlorpromazine.

Considering that (*a*) the routine assay is based on only a 3-ml blood sample instead of 10 ml, (*b*) the usual therapeutically effective and clinically used dose of chlorpromazine is in the range of 150–600 mg/day (8), and (*c*) the steady-state blood levels[3] of the drug in a large number of patients receiving total daily oral doses of 200 and/or 600 mg/m^2 range from only 21 to 110 ng/ml, it appears reasonable that the interference due to these promazine metabolites, if present in other patients as well, may not be of major concern. However, this issue is being evaluated further.

Analysis of Unknowns—To determine the accuracy of the quaternization method when applied to blood or plasma, spiked samples were prepared and subsequently blind analyzed to determine the chlorpromazine and its sulfoxide concentrations. Table IV lists the results.

Patient Data—The developed and standardized assay methodology is currently being used in chronic clinical studies with schizophrenic patients aimed at correlating the orally given dose of chlorpromazine with its various pharmacokinetic parameters as well as the clinical responses observed. Figure 1 shows the absorption–elimination time profiles of chlorpromazine and its sulfoxide in a patient (C.D.) following ingestion of a single oral dose of 120 mg/m^2 of chlorpromazine (syrup formulation) on an empty stomach during Weeks 0, 4, and 12 of a chronic study.

The elimination of chlorpromazine in the blood of the same patient appeared to follow first-order kinetics, with a half-life of 3–4 hr and the elimination rate constant, k_e, of 17.3–22.3 × 10^{-2} hr^{-1} (Fig. 2). The elimination rate was significantly ($p < 0.05$) higher at Week 12 than at Week 0, suggesting a possible autoinduction caused by chlorpromazine and/or its metabolite(s) in the patient. However, this difference in half-lives may have been due to an intrasubject variation with time.

Application to Generic Bioequivalence—The full potential of

[3] Determined in this laboratory.

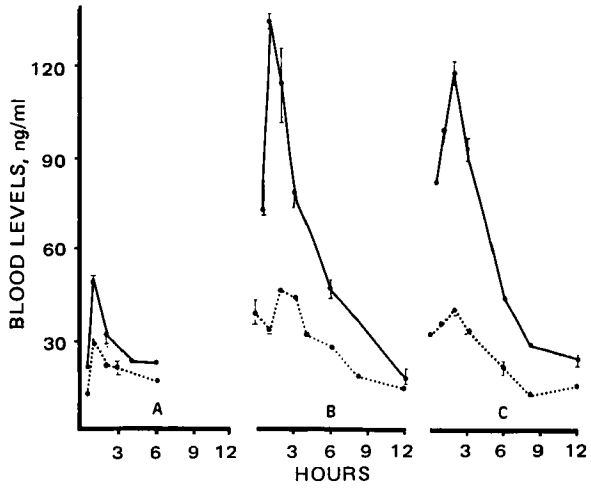

Figure 1—*Appearance and disappearance of chlorpromazine (——) and its sulfoxide (. . . .) in the blood of a chronic schizophrenic patient receiving 600 mg/m^2/day in four equally divided doses. The samples were drawn before and after giving the single oral kinetic dose (120 mg/m^2) of chlorpromazine on Weeks 0 (A), 4 (B), and 12 (C). Each point represents the mean of two values* ±SD.

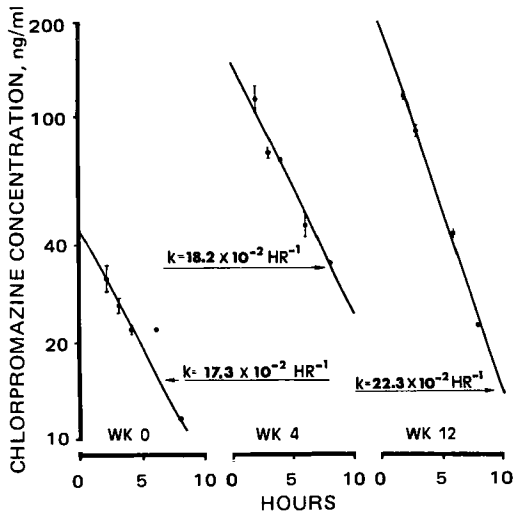

Figure 2—*Semilogarithmic plots of the data on elimination of chlorpromazine in a chronic schizophrenic patient receiving 600 mg/m^2/day in four equally divided doses. The blood samples were drawn before and after giving the single oral kinetic dose (120 mg/m^2) of chlorpromazine on Weeks 0, 4, and 12. Each point represents the mean of two values* ±SD.

the developed assay method may be gauged by realizing that it is applicable to many tertiary amine drugs other than chlorpromazine. Furthermore, assessment of the bioavailability of generic products that require an accurate and precise method of analysis makes this assay methodology valuable, particularly for drugs that show a large volume of distribution in the body. So far, atropine, amitriptyline, chlorprothixene, codeine, imipramine, morphine, pilocarpine, strychnine, and trifluoperazine have been found to react with 9-bromomethylacridine and produce fluorescent products upon photolysis. The stoichiometry of some of these reactions was found to hold over a wide range of concentrations. For example, Fig. 3 shows a concentration–fluorescence correlation with amitriptyline, chlorprothixene, and imipramine.

In a global cooperative assay study, 14 different laboratories were sent blind spiked plasma samples containing low and high concentrations of chlorpromazine and its sulfoxide. Of all the returns of data, the acridine fluorometric methodology appeared unmatched in terms of accuracy and precision (9).

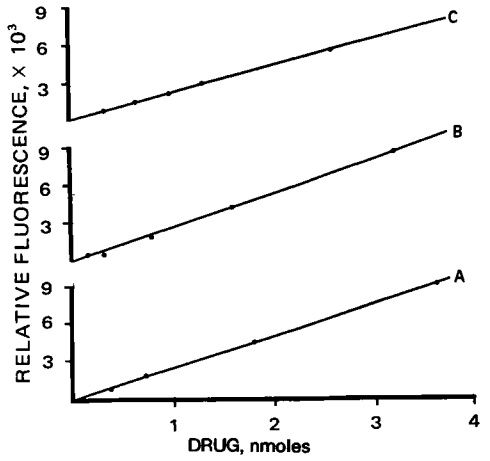

Figure 3—*Stoichiometric correlation of the direct reaction of imipramine (A), chlorprothixene (B), and amitriptyline (C) with 9-bromomethylacridine. Each point is the mean of three values.*

The described assay methodology has shown a specificity for tertiary amines, which precludes the possibility of interference due to the primary and secondary amine metabolites generally present in the blood of patients receiving drugs such as chlorpromazine (6, 7, 10) and amitriptyline (11, 12). However, the methodology does not presently discriminate between mixtures of tertiary amine drugs such as chlorpromazine and chlorprothixene. Nonetheless, it should be possible to differentiate the quaternary products of these drug mixtures by developing new TLC systems capable of such separations or by exploring electrophoresis prior to the photolytic step. These and other approaches are being attempted.

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Effects of Various Hydrodynamic Conditions on Dissolution Rate Determinations

FRED L. UNDERWOOD * and DONALD E. CADWALLADER ˣ

Abstract □ An automated potentiometric procedure was used in dissolution rate studies to determine the effects of various hydrodynamic conditions on dissolution rate determinations. Changes in the hydrodynamics of the system resulted from using various sizes and shapes of dissolution vessels. Dissolution rate constants for benzoic acid prills in distilled water at pH-stat 6.2 were used as a measure of the agitation intensities present in the different shaped vessels. Great variations in the dissolution rates occurred in vessels with the same diameter and stirrer blade position when the shapes of the bottom of the vessel were varied. A similar order of dissolution rates was obtained at 100 and 150 rpm for the individual vessels at various propeller heights. The order differed from one vessel to another, depending on the shape of the bottom (concave, convex, or flat) of the vessel. In some cases, a change in the type of bottom resulted in the opposite order of rates for vessels with the same diameter.

Keyphrases □ Dissolution rate—benzoic acid, effect of varying hydrodynamic conditions by varying sizes and shapes of dissolution vessels □ Hydrodynamic conditions—effect on dissolution rate of benzoic acid by varying sizes and shapes of dissolution vessels □ Benzoic acid—dissolution rate, effect of varying hydrodynamic conditions by varying sizes and shapes of dissolution vessels

For several years, it has been recognized that the availability of a drug for GI absorption from solid dosage forms is often reflected by *in vitro* dissolution rates. This observation has stimulated research in dissolution rate studies. Many different variables, *e.g.*, particle size of dissolving substance, agitation intensity, temperature, and size and shape of the dissolution vessels can influence the dissolution rate of a substance.

Noyes and Whitney (1) made the first quantitative study of the dissolution process and derived an equation relating the dissolution rate to the surface area of the dissolving material. A relationship between dissolution rates and agitation intensity has been known to exist for some time, but relatively few studies have been carried out to show how various hydrodynamic conditions that can influence the agitation can affect the dissolution rate of a drug. The agita-