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High-Pressure Liquid Chromatographic Determination of Promethazine Plasma Levels in the Dog After Oral, Intramuscular, and Intravenous Dosage

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Abstract □ Plasma levels of promethazine were determined using a high-pressure liquid chromatographic procedure incorporating a fixed wavelength (254 nm) UV detector, following single 50-mg intravenous, intramuscular, and oral doses to two male dogs. Initial plasma promethazine concentrations following intravenous doses were 556 and 535 ng/ml in the two dogs. The subsequent decline in drug levels were satisfactorily described by a triexponential function. Peak promethazine levels of 76 and 64 ng/ml were obtained 0.5 hr following intramuscular doses. Peak levels for the oral doses were 10.6 and 11.0 ng/ml occurring 2 hr after dosing. The apparent biological half-life of promethazine, obtained from only 2–3 data points, varied from 8.5 to 27.7 hr. Areas under the promethazine plasma curves, compared to values obtained from intravenous doses between 0 and 24 hr, indicated that systemic availability of intact drug was 55–73% following intramuscular injection and 8.3–9.5% following oral administration.

Keyphrases □ High-pressure liquid chromatography—determination of promethazine plasma levels after oral, intramuscular, and intravenous dosage, dogs □ Promethazine—high-pressure liquid chromatographic determination of plasma levels after oral, intramuscular, and intravenous dosage, dogs □ Pharmacokinetics—high-pressure liquid chromatographic determination of promethazine plasma levels after oral, intramuscular, and intravenous dosage, dogs

Promethazine has been used extensively for the control of allergy and as a sedative and antiemetic; however, little information is available on its bioavailability from oral dosage forms or on its pharmacokinetics. This has been due to the absence of suitable methods for determining promethazine in body fluids.

Gas chromatographic procedures have been used to measure promethazine (1, 2) and other phenothiazines (3, 4), but these methods lack sensitivity and require extensive sample preparation before chromatography. Studies in this laboratory showed that unless promethazine is separated from its various metabolites prior to gas chromatography, spurious results may result from on-column reduction of oxidized metabolites to the parent compound (5).

High-pressure liquid chromatographic (HPLC) procedures for promethazine have been reported recently (6–8). Two of the procedures (6, 7) require either large samples or prior derivatization, while the other (8) requires the use of electrochemical detection. The lack of assays suitable for routine use is the major cause for the scarcity of information on the pharmacokinetics of promethazine in animals (2, 9) and humans (1, 10) in the literature.

The use of a specific and sensitive HPLC assay to measure plasma promethazine levels following single oral, intramuscular, and intravenous doses to male dogs is described. Preliminary details of the liquid chromatographic procedure and comparisons with a gas chromatographic assay have been described elsewhere (5).

EXPERIMENTAL

Assay for Promethazine in Plasma—To 2 ml of plasma were added 200 μl of a 0.56-μg/ml aqueous solution of chlorpromazine hydrochloride (equivalent to 0.5 μg/ml of chlorpromazine) as internal standard, 0.5 ml of 1.0 *N* sodium hydroxide, and 8 ml of methylene chloride. After shaking for 15 min at low speed on a horizontal shaker and centrifuging at 3000×*g* for 5 min, the upper aqueous phase was discarded by aspiration. The organic phase was transferred to a clean tube and evaporated to dryness under nitrogen at room temperature. The tube was rinsed with 1 ml of methylene chloride and was again evaporated to dryness. The residue was reconstituted in 60 μl of the chromatographic mobile phase by vortexing, centrifuged at 3000×*g* for 1 min, and 20 μl of the supernate was injected into the chromatograph.

The HPLC consisted of a solvent pump[1]; a fixed-volume (20 μl) injection valve[2]; a 10-μm particle size, reversed-phase octadecyl column[3]

[1] Model 110, Altex Scientific, Berkeley, Calif.
[2] Rheodyne sample injector.
[3] Lichrosorb C-18 reversed-phase column, Altex Scientific, Berkeley, Calif.

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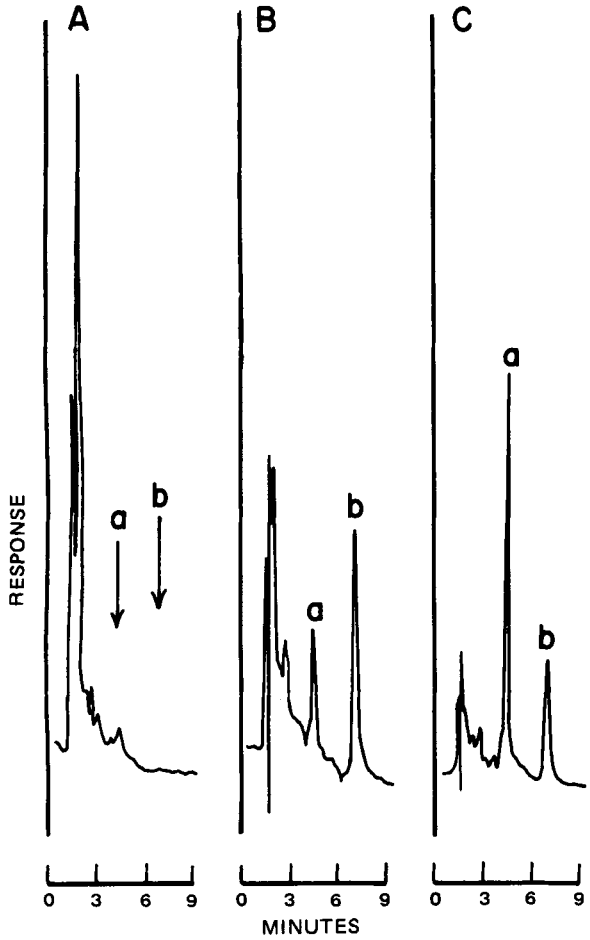

Figure 1—*Chromatograms obtained from (A) plasma containing no drugs; (B) a plasma sample obtained 12 hr following intramuscular injection, containing 9.1 ng/ml promethazine (a) and 50 ng/ml chlorpromazine (b); and (C) a plasma sample obtained 2 hr following intramuscular injection, containing 60 ng/ml promethazine (a) and 50 ng/ml chlorpromazine (b).*

(4.6 mm × 25 cm); a precolumn[4]; and a fixed wavelength (254 nm) detector[5]. Chromatograms were recorded on a chart recorder[6] with 10-mV input and 20-cm/hr chart speed. The mobile phase consisted of 42% acetonitrile and 3% *n*-nonylamine in 0.02 *M* phosphate buffer, pH 2.5. The flow rate was 2 ml/min at a pump pressure of 2.0×10^3 psi.

Dog Study—Two 3-year-old, mixed-breed, beagle-type dogs weighing 16 and 17 kg were fasted overnight before receiving 50-mg doses of promethazine orally[7], and by intramuscular[8] and intravenous[8] injection on three separate occasions at least 1 week apart. (Doses were administered at 8 am.)

During each experiment the dog was placed in a restraining sling, designed so that the dog could stand or rest comfortably but could not displace indwelling catheters which were placed into leg veins. To familiarize the dogs with this procedure, they were placed in the apparatus for 6 hr during each of the 3 days preceding the study.

Tablet doses (2 × 25 mg) were placed on the back of the dogs' tongues, so that the tablets were not fractured or chewed before being swallowed. This was immediately followed by 50 ml of water administered *via* an irrigating syringe[9]. Intramuscular doses were administered in 1 ml of physiological saline into a hind-leg thigh muscle. Intravenous doses were administered in 1 ml of physiological saline into a hind-leg vein over a 30-sec period.

Following the oral dose, blood samples (7–8 ml) were taken immediately before, at 30 min, and at 1, 2, 3, 4, 6, 8, 12, and 24 hr after dosing. An additional blood sample was drawn 15 min after the intramuscular dose, while additional samples were drawn at 5, 10, 15, and 45 min following the intravenous dose. During the initial 6-hr sampling period, blood samples were obtained *via* a vein infusion set[10] positioned in the front leg. The infusion set was kept patent by means of an intravenous drip (4 drops/min) of normal saline[11] containing 1 U/ml of sodium heparin[12]. At each sampling time 2 ml of residual fluid were withdrawn from the infusion set before drawing a 7–8-ml blood sample into a clean syringe[13]. The sets were removed after 6 hr and the dogs were released from the

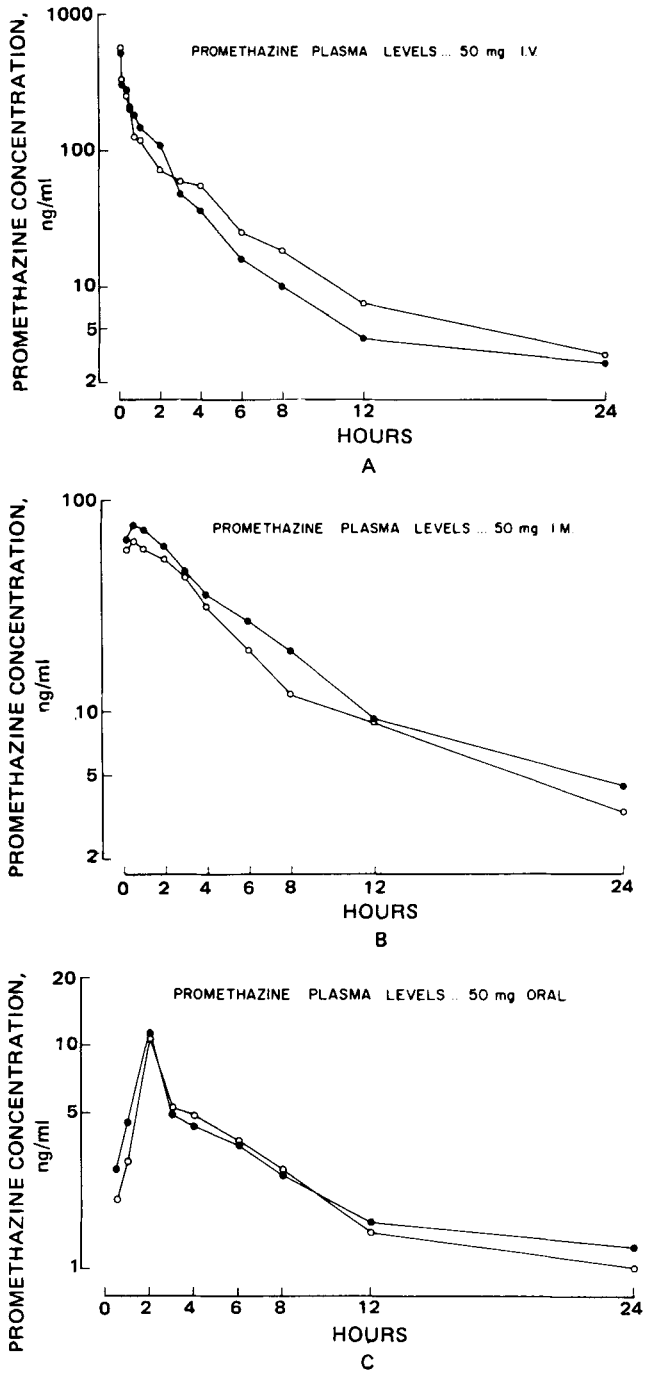

Figure 2—*Promethazine plasma levels in Dog 1 (○) and Dog 2 (●) following a single 50-mg (A) intravenous, (B) intramuscular, or (C) oral dose.*

[4] CO:PELL ODS, 30-38 m. Whatman Inc., Clifton, N.J.
[5] Model 153, Altex Scientific, Berkeley, Calif.
[6] Model 023, Perkin-Elmer Instrument Division, Norwalk, Conn.
[7] Phenergan, 25-mg tablets, Lot 1800131, Wyeth, Philadelphia, Pa.
[8] Promethazine Hydrochloride Injection, Lot 80B023, 50 mg/ml, Geneva Generics, Bloomfield, Col.
[9] Pharmaseal Labs., Glendale, Calif.
[10] Miniset, vein infusion set with winged adaptor, Travenol Laboratories, Deerfield, Ill.
[11] Sodium chloride 0.9% irrigating solution, USP, Travenol Laboratories, Deerfield, Ill.
[12] Panheprin, Lot 23795 AF, 1000 USP U ml^{-1}, Abbott Laboratories, North Chicago, Ill.
[13] B-D 10-cc syringe Luer Slip Tip, Becton, Dickinson and Co., Rutherford N.J.

Table I—Day-to-Day Reproducibility of the Assay for Promethazine in Plasma[a]

Concentration in Plasma, ng/ml	Found, ng/ml	Recovery, %	Coefficient of variation, %
1	1.1	110	10.8
2.5	2.54	102	8.4
5	5.21	104	6.8
10	10.2	102	5.7
25	24.7	99	5.9
50	47.6	95	5.3
75	71.8	96	1.8
100	103.0	103	2.1
150	149.6	100	2.0
200	196.6	98	4.5
250	252.3	101	2.3

[a] n = 10 for all concentrations.

Table II—Retention Times of Various Phenothiazines

Phenothiazine Compound	Retention Time, min
Promethazine-5-sulfoxide	2.4
Promethazine	4.5
Promazine	4.5
Chlorpromazine	7.2
Triflupromazine	9.0
Thioridazine	10.5

apparatus. Subsequent blood samples were obtained in heparinized tubes[14]. The samples were drawn immediately, and before the blood came into contact with the rubber tube stoppers, they were replaced by cork stoppers wrapped in aluminum foil. Plasma was separated from blood by centrifugation and stored at −20° until assayed. All samples were assayed within 2 weeks of collection.

Reagents—Human plasma for assay development was purchased[15]. Dog plasma for assay validation in this species was obtained from dogs in the School of Pharmacy animal care facility. Promethazine hydrochloride[16], promethazine-5-sulfoxide hydrochloride[16], and chlorpromazine hydrochloride[17], of reference standard quality; acetonitrile[18], methylene chloride[18], toluene[19], potassium phosphate monobasic[19], of analytical grade quality; and n-nonylamine (98%)[20] and hexamethyldisilazane[21], as supplied were used. All glassware was silanized with 5% hexamethyldisilazane in toluene prior to use.

RESULTS

Assay for Promethazine in Plasma—Typical chromatograms obtained from dog plasma containing no added compounds and from plasma containing promethazine and chlorpromazine (internal standard) are shown in Fig. 1. Retention times for promethazine and chlorpromazine were 4.5 and 7.0 min, respectively. Assay response was linear for promethazine concentrations between 1 and 250 ng/ml. The coefficient of variation in assay response was 11% for a promethazine concentration of 1 ng/ml, and was <10% for all other concentrations. The linear regression of the promethazine–chlorpromazine peak height ratios against promethazine concentrations from repeated determinations was $y = 0.013 + 0.053\,x$, $r = 0.999$, $n = 100$. The day-to-day reproducibility of the procedure, determined during the assay of actual plasma samples, is shown in Table I. The extraction efficiency for promethazine was 94.5 ± 5.6% (n = 5). Substituting dog plasma for human plasma did not affect the assay results.

The use of n-nonylamine in the liquid chromatographic mobile phase has the effect of reducing compound retention time and improving the peak shape on chromatograms (11). This modification improves assay sensitivity. Promethazine-5-sulfoxide, a major metabolite of promethazine, has a retention time of 2.5 min under the chromatographic conditions used, and any peak resulting from the presence of this metabolite was obscured by endogenous plasma components (5). The retention times of some phenothiazines in the system are shown in Table II.

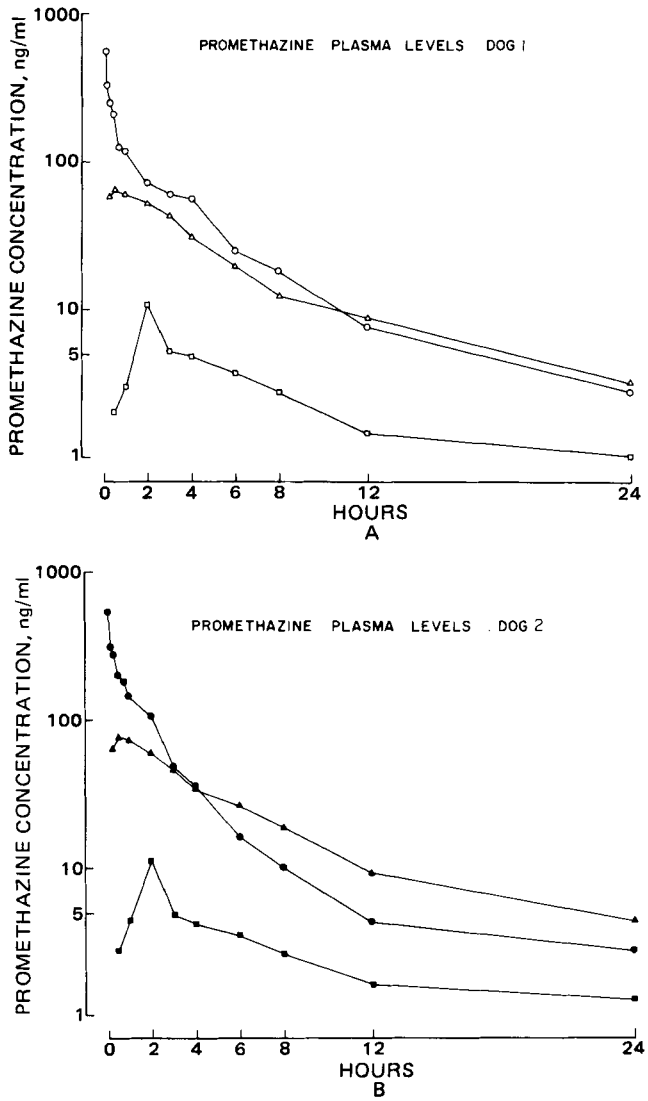

Figure 3—*Promethazine plasma levels following (A) single 50-mg intravenous (○), intramuscular (△), and oral (□) doses to Dog 1; and (B) single 50-mg intravenous (●), intramuscular (▲), and oral (■) doses to Dog 2.*

Pharmacokinetic Study in Dogs—Preliminary studies showed that the rubber stoppers, supplied with the evacuated glass tubes[14], reduce the apparent promethazine concentration by 25–30% compared with values obtained when aluminum-wrapped cork stoppers are used. Plasticizers in the rubber stoppers previously have been shown to displace chlorpromazine from plasma protein binding sites with subsequent redistribution of drug from plasma water into erythrocytes (12). Although it has not been shown that a similar phenomenon occurs with promethazine, the good assay results obtained with the aluminum cork stoppers justified their use.

The plasma promethazine profiles following single 50-mg oral, intramuscular, and intravenous doses of promethazine (hydrochloride) are shown in Fig. 2. The profiles obtained from the three dosage routes are combined for each dog for comparison in Fig. 3.

The promethazine plasma levels were similar in the two dogs. Concentrations of 556 and 535 ng/ml were obtained 5 min after the intravenous doses. Plasma levels declined rapidly at first and then more slowly to reach values of 3–4 ng/ml at 24 hr. The decline in plasma levels appeared to be triphasic in nature. Graphical analysis of the individual data sets in terms of a triexponential function gave rise to half-lives of 0.18, 1.9, and 8.3 hr and 0.14, 1.4, and 27.7 hr from the three exponents in Dogs 1 and 2, respectively. The coefficients of determination [$r^2 = (\Sigma obs^2 - \Sigma dev^2)/\Sigma obs^2$] between observed and predicted promethazine plasma concentrations using the triexponential function were 0.9996 and 0.9997.

[14] Green Stoppered Vacutainer, evacuated glass tubes coated with 143 U of sodium heparin, Becton-Dickinson, Rutherford, N.J.
[15] American Red Cross, Madison, Wis.
[16] Wyeth Laboratories, Inc., Philadelphia, Pa.
[17] Smith Kline & French Laboratories, Philadelphia, Pa.
[18] Burdick & Jackson, Muskegon, Mich.
[19] Fisher Scientific Co., Fair Lawn, N.J.
[20] Aldrich Chemical Co., Milwaukee, Wis.
[21] Pierce Chemical Co., Rockford, Ill.

Peak promethazine levels of 76 and 64 ng/ml were obtained at 0.5 hr following the intramuscular doses; levels declined rapidly and then at a slower rate after 8–12 hr. At 24 hr the levels from these were similar to those observed following intravenous injection. The terminal half-lives, which were calculated from 12- and 24-hr data points in one dog, and the 8–24-hr data points in the other, were 8.5 and 11.6 hr.

Two hours following the administration of oral doses, peak promethazine levels of 10.6 and 11.0 ng/ml were obtained. The subsequent decline in drug levels was again nonlinear, and the drug was barely detectable in plasma at 24 hr. The later times of peak promethazine levels after oral doses, compared to intramuscular doses, indicates that absorption is slower *via* the oral route.

The efficiency of drug absorption from intramuscular and oral doses can be calculated by comparison of areas under plasma curves resulting from the different doses. The trapezoidal areas under plasma curves for Dog 1 in a 24-hr period were 714, 391, and 59 ng hr/ml following intravenous, intramuscular, and oral doses, respectively. In Dog 2 the equivalent values were 650, 472, and 62 ng hr/ml. Since the same dosage was given by each route, the absorption efficiency of unchanged promethazine can be obtained by direct comparison of area values. Thus, compared with intravenous values, drug bioavailability was 55 and 73% after intramuscular doses, and after oral doses, 8.3 and 9.5%.

DISCUSSION

The assay for promethazine used in this study is suitable for routine laboratory use. Sample preparation is uncomplicated and compounds are measured using a fixed wavelength UV detector. The method is sufficiently sensitive and specific to monitor plasma promethazine levels in animals and humans during a 24-period following therapeutic doses (5).

The reduction in plasma promethazine levels observed when rubber stoppers were used in blood collection tubes is similar to that reported previously for chlorpromazine (12). Failure to account for this may give rise to considerable assay error. The present data do not establish, however, that the mechanism causing reduced promethazine levels is the same as that for chlorpromazine. As a result, protein-binding characteristics of promethazine and other phenothiazines under different situations are being examined.

The plasma promethazine levels obtained in the two dogs show that the pharmacokinetics of promethazine are complex, and its disposition in the body cannot be described in terms of a simple one- or two-compartment kinetic model. It cannot be determined from these data whether the apparent triphasic decline in plasma levels following intravenous doses is due to differential uptake of drug by deep and shallow tissue compartments or to concentration-dependent changes in drug binding to plasma proteins.

Comparison of the areas under plasma curves from the three dosage routes indicates that the overall bioavailability of intact promethazine is reduced somewhat after intramuscular injection compared to intravenous administration. This is not uncommon (13) and probably results from partial degradation of drug at the intramuscular injection site and from slow release of some drug from the site at later, postsampling times.

The marked reduction in promethazine plasma levels following oral doses, compared to intravenous and intramuscular doses, indicates inefficient absorption by this dosage route. The degree of reduction is greater than that previously reported in rabbits (9), where systemic drug availability was calculated to be 50%, but appears to be similar to that reported in a single human volunteer following 12.5-mg intravenous and oral doses (14).

Although extensive first-pass metabolism of oral promethazine has been inferred (10, 14), the data currently available from this and previous studies is insufficient to determine the relative contributions of limited absorption and first-pass metabolism to the poor systemic availability of oral promethazine.

As the availability of poorly absorbed drugs is likely to be more variable, and more easily influenced by other factors, than that of efficiently absorbed compounds, the results obtained in this study confirm that promethazine is a compound with potential bioequivalence problems (15). The data presented here and elsewhere (10, 14) indicate that the dog may be a suitable animal model for promethazine bioavailability studies.

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