Blood Levels of Chlorphentermine in Man

HUNG WON JUN and EDWARD J. TRIGGS

Abstract □ Blood level determinations of chlorphentermine (*p*-chloro-α,α-dimethylphenethylamine) in man following oral administration of the drug in solution, as a prolonged-release formulation, and as an intravenous injection have been carried out by gas-liquid chromatography (GLC). The drug had a long apparent elimination half-life in the body, and the therapeutic need for a prolonged-release formulation of a drug of this type therefore may be doubtful. The significance of blood level studies in evaluating therapeutic dosage levels was emphasized with respect to dosage form evaluation and pharmacokinetics, even though such studies may not represent pharmacological efficacy in cases of extensive drug localization.

Keyphrases □ Chlorphentermine blood levels—determination □ Pharmacokinetic parameters—chlorphentermine distribution, elimination □ Half-life, apparent—chlorphentermine □ GLC—analysis

Urinary excretion studies of amphetamine analogs have been carried out by a number of workers as a method for evaluating biopharmaceutical drug parameters in man (1, 2). This technique, however, is not completely satisfactory as a number of assumptions are implicit in the method (3, 4). To overcome some of these shortcomings, blood level studies have been developed for a number of these compounds (5, 6). Such studies require the use of specific and sensitive analytical procedures since these drugs are extensively concentrated extravascularly in the body (7). Both the use of radioisotopically labeled drugs (8) and electron-capture GLC (9) have been found satisfactory in such analysis.

A method to determine chlorphentermine blood levels in man was developed using flame ionization GLC, and a study made of the drug following oral administration of solution and prolonged-release formulations together with intravenous administration.

EXPERIMENTAL

Extraction Procedure and Analytical Method—The extraction procedure from blood was based on that used by Reynolds and Beckett (10) for a series of local anesthetics. A 3–5-ml. blood sample was diluted with an equal volume of water in a 15-ml. glass centrifuge tube, and 1 ml. of 20% w/v NaOH was added. Four milliliters of redistilled reagent grade diethyl ether was then added. The tube was stoppered tightly and shaken for 10 min., followed by centrifugation at 2000 r.p.m. for 5 min. The upper ethereal layer was transferred to a second centrifuge tube, and the procedure was repeated for two further ether extractions. The combined ethereal extracts were shaken twice for 10 min with 3 ml. 0.1 *N* HCl. The aqueous layer containing the extracted drug was transferred to a third centrifuge tube and adjusted to approximately pH 11 with 20% w/v NaOH (about 1 ml.) and extracted 3 times with 3 ml. diethyl ether as for blood. The ethereal layers were transferred to a 12-ml. glass sedimentation tube containing 1 ml. of a 2 mcg./ml. solution of azobenzene in diethyl ether (internal marker).

The sedimentation tube was placed in a water bath at 40° and the contents evaporated to near dryness. The tube was then stoppered and placed in an ice bath to produce a final volume of about 5 μl. A 2–3-μl. sample of this concentrate was then injected onto the gas chromatographic column.

The apparatus and conditions for GLC were as reported by Jun and Triggs (11) with the following modifications: (*a*) the gas chromatographic column was packed with 1% silicon gum rubber[1] on 80–100-mesh general-purpose solid support[2], and (*b*) helium flow rate 40 ml./min.

Blood Level Studies—*Oral Administration*—Chlorphentermine hydrochloride was administered in 20 ml. aqueous solution as a 100-mg. dose (5 mg. HCl/ml.) to four healthy male subjects (aged 25–30 years), approximately 30 min. after a light breakfast of coffee and toast. Blood samples (7 ml.) were taken intravenously using Vacutainer tubes[3] at 30-min. intervals for 3 hr. and then at 7, 11, 13, 25, 35, and 48 hr. after drug administration. Urine samples were also collected irregularly over the 48-hr. time period, and in some cases for a further 8 days.

The drug was also administered as a 78-mg. dose in the form of prolonged-release Preparation A[4] to three of the four subjects who had received the drug in solution. Blood samples were taken as previously at 60-min. intervals for 4 hr.; 120-min. intervals for a further 8 hr.; and at 24, 28, 32, 36, and 48 hr. after drug administration. Urine samples were taken as previously described.

Intravenous Administration—Chlorphentermine hydrochloride injections were prepared in the laboratory and sterilized by passing the solution through a bacterial filter[5] under aseptic conditions followed by autoclaving. The solution for injection contained 25 mg./ml. of the drug as the hydrochloride salt. The ampuls were tested for sterility[6] and for drug content by GLC.

The drug was infused intravenously over a 2-min. period as a 50-mg. dose to two of the four subjects. Blood samples were taken at 2, 5, 10, 20, 40, 60, and 120 min. and at 4, 7, 10, 24, 36, and 48 hr. after drug administration. Urine samples were taken as previously described.

RESULTS

Extraction Procedure and Analytical Method—Figure 1 shows a typical chromatogram of a blood extract containing chlorphentermine. Good symmetrical peaks were obtained for both chlorphentermine and the internal marker, azobenzene. Analysis of blank samples of blood from the subjects used in the studies showed no interfering peaks with the same retention times as chlorphentermine or azobenzene.

Figure 2 illustrates a calibration curve obtained for chlorphentermine in blood; the ratio peak height of chlorphentermine to peak height of azobenzene was plotted against the concentration of chlorphentermine per 5 ml. of blood. The calibration curve was a straight line passing through the origin.

Ten duplicate analyses of a blood sample containing 0.5 mcg. chlorphentermine/5 ml. were performed, and the results showed a standard deviation of ±4.92%.

The percentage recovery of chlorphentermine from blood was found to be 95 ± 5%.

Blood Level Studies—*Oral Administration*—Average blood levels for chlorphentermine following oral administration of the 100-mg. solution dose to four subjects are shown in Fig. 3. The pharmacokinetic parameters, apparent elimination half-life, apparent volume of distribution, and area under the blood level curve were estimated and appear together with the cumulative urine data in Table I.

Subjects experienced no stimulant effect from the drug at this dosage level. The only effects noticed were pupil dilation and marked anorexia.

[1] OV-1 Hewlett Packard Ltd.
[2] Chromosorb G(AW-DMCS), Hewlett Packard Ltd.
[3] Becton, Dickinson & Co., Canada, Ltd.
[4] Commercially available preparation of chlorphentermine hydrochloride.
[5] Standard Millipore filter type HA, B.D.H. (Canada) Ltd.
[6] USP XVII Sterility Test for injection.

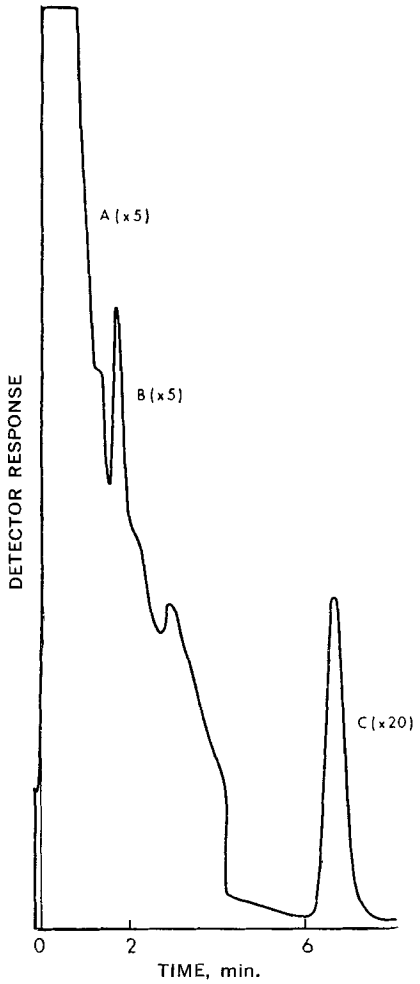

Figure 1—*Typical chromatogram of blood extract containing chlorphentermine. Key:* A = *solvent peak (diethyl ether);* B = *chlorphentermine;* C = *internal marker (azobenzene). Figures in parentheses signify attentuation.*

Average blood levels for chlorphentermine following oral administration of the 78-mg. prolonged-release formulation to three subjects are shown in Fig. 4. Areas under the blood level curves and urine data are shown in Table I. No subjective effects were noted.

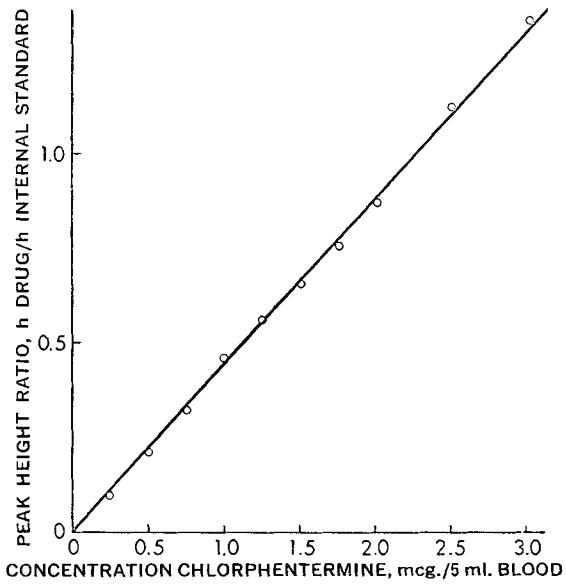

Figure 2—*Calibration curve for chlorphentermine in blood.*

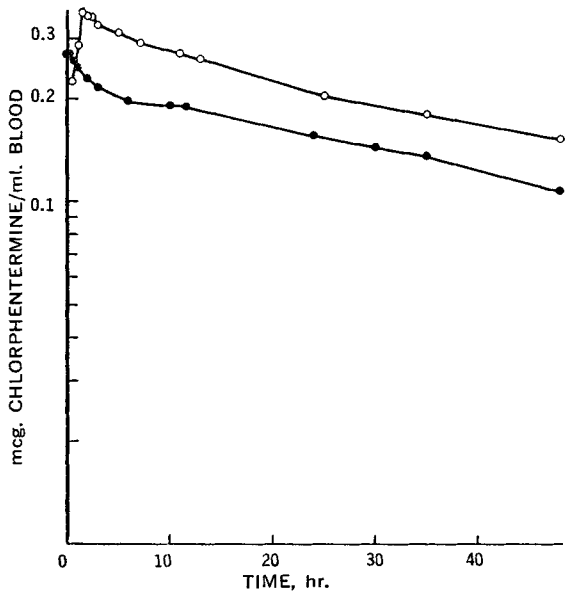

Figure 3—*Average blood level-time curves for chlorphentermine following drug administration in solution (100 mg.) and intravenous injection (50 mg.). Key:* ○, *solution; and* ●, *intravenous injection.*

Intravenous Administration—Average blood levels of chlorphentermine following intravenous administration of the drug to two subjects as a 50-mg. dose are shown in Fig. 3. The pharmacokinetic parameters and urine data appear in Table I.

Effects noted were lightheadedness and marked anorexia.

DISCUSSION

In most chromatograms the drug peak occurred on the solvent slope at the low attentuations used in these studies; however, reasonably accurate peak height measurements could be made as indicated by the size of the standard deviation. Drug concentrations as low as 125 ng./5 ml. of blood could be detected. The average blood levels for chlorphentermine (see Fig. 3) were well within the range of sensitivity of the method.

Peak blood levels for chlorphentermine in all subjects occurred at 1.5–2.5 hr. following oral administration of the drug in solution, and blood levels after the peak showed a slow exponential decline. The apparent elimination half-life for the drug ranged from 35 to 45 hr. (average 41 hr.) and was in close agreement with that obtained from predicted blood levels determined by analog computation (3). The apparent volume of distribution (average 213 l.), together with the long apparent elimination half-life, suggested extensive tissue

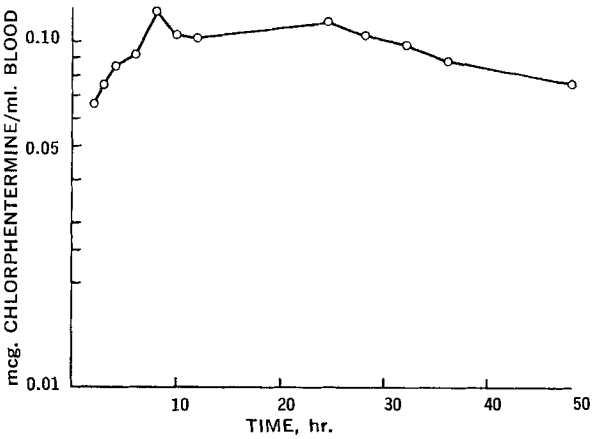

Figure 4—*Average blood level-time curve for chlorphentermine following drug administration as prolonged-release formulation A (78 mg.).*

Table I—Some Pharmacokinetic Parameters for Chlorphentermine following Solution, Prolonged-Release, and Intravenous Drug Administration in Man

Subject[a]	Mode of Administration	Dose, mg. HCl	Apparent Elimination Half-Life, hr.	Apparent Volume of Distribution[b]	Area under Blood Level Curve, mcg. hr./ml.[c]	% Dose Excreted Unchanged in Urine in 48 hr.
H.J.	Solution	100	45	185	14.5	23.3
D.S.	Solution	100	35	214	10.7	25.8
W.J.	Solution	100	45	298	8.6	19.6
K.M.	Solution	100	40	245	8.5	16.1
H.J.	i.v.	50	42	139	8.7	20.4
D.S.	i.v.	50	44	198	6.6	21.6
H.J.	Prolonged-release	78	—	—	4.5	16.4
K.M.	Prolonged-release	78	—	—	5.8	12.3
W.J.	Prolonged-release	78	—	—	3.3	14.1

[a] Incompleted crossover study. [b] See *References 1* and *21*. [c] Calculated by means of trapezoid rule (20).

distribution of the drug. These findings suggested that the drug may be localized to a large extent in the body. Previous reports (12, 13) have shown chlorphentermine to be localized in various body organs of rats and mice, notably brain and lung tissue.

Peak blood levels for chlorphentermine occurred at 6–8 hr. following oral administration of prolonged-release Preparation A, suggesting delayed release and subsequent slow absorption of the drug. The areas under the blood level curves were compared following intravenous and prolonged-release drug administrations, and an average of 62% (maximum 87%) of the drug was found to be available from the prolonged-release dosage form. However, it is possible that this average result may be an underestimate since comparisons were made only over the 48-hr. time period and ideally should be compared from zero to infinite time (22). Subject variation, which did occur (see Table I), probably also contributed to the spread of the value of the availability term.

Urinary excretion studies of chlorphentermine in this and previous studies (3) suggested almost complete drug availability from this prolonged-release preparation. Thus, the determination of drug availability from urinary excretion studies may lead to a discrepancy as the assumption is made that urine drug levels reflect tissue levels of the drug which might not be the case where some degree of localization occurs in the body.

The observations that the apparent elimination half-life of the drug was, on the average, in excess of 40 hr. and that the cumulative amount of drug in the urine in 48 hr. was less than 25% of the administered dose suggested doubts as to whether a prolonged-release drug formulation was indeed clinically necessary. It could, however, be argued that tissue levels resulting from administration of a prolonged-release formulation would perhaps follow a more clinically acceptable pattern than those levels following administration of the drug in solution. This possibility and the determination of pharmacokinetic data are the subject of further study. It has been shown previously that this particular prolonged-release formulation gives a good *in vivo/in vitro* correlation of drug availability (3).

A dose of 50 mg. of drug was chosen for the intravenous drug administration studies since it was felt that there would be less subjective effects at this dosage level. The initial phase of the blood level curve following intravenous drug administration was indicative of the extensive extravascular distribution of the drug (see Fig. 3). However, it was not possible with the blood sampling times used to extrapolate accurately this initial drug distribution phase to time zero. Reference to the area under the blood level curves (see Table I) following oral and intravenous drug administration showed that the drug was completely available when administered in solution. This confirmed results in an earlier study in which the cumulative amount of drug excreted in acid pH controlled urine over an extended period of time following drug administration in solution was about 90% (14). Also, previous reports have shown the drug to be almost completely excreted unchanged (15, 16).

It is difficult to determine the required therapeutic dose levels of a drug which is largely localized in the tissues by means of blood level studies. Ideally the concentration of drug at its site of action should be measured or a pharmacological response of the drug monitored. However, blood level studies are of importance as they enable the determination of drug pharmacokinetic parameters (17) and comparison between performance of different dosage forms to be made (18, 19).

SUMMARY

1. Blood level determinations of chlorphentermine in man following administration of the drug in solution, prolonged-release formulation, and as an intravenous injection have been carried out by GLC.

2. The apparent elimination half-life of the drug in man was on the average 41 hr.

3. Comparison of areas under the blood level–time curves for intravenous and oral solution drug administration indicated complete availability of the drug from solution. However, the area under the curve resulting from administration of the prolonged-release formulation was lower possibly due to the limited experimental time period and subject variation.

4. The shape of the blood level–time curves suggested that the drug undergoes some degree of body localization.

5. Therapeutically, prolonged-release formulations of chlorphentermine may be doubtful.

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ACKNOWLEDGMENTS AND ADDRESSES

Received August 15, 1969, from the *Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.*

Accepted for publication October 8, 1969.

The investigation was supported by a research grant MRC-MA-3323 from the Medical Research Council of Canada.

The authors would like to thank Warner-Chilcott Labs. (Canada) Ltd., for generously supplying them with chlorphentermine hydrochloride and for their help in obtaining volunteers for our blood level studies.

Thanks are also expressed to volunteers D. S., W. J., and K. M.

Pharmacokinetic Profile of Coumermycin A_1

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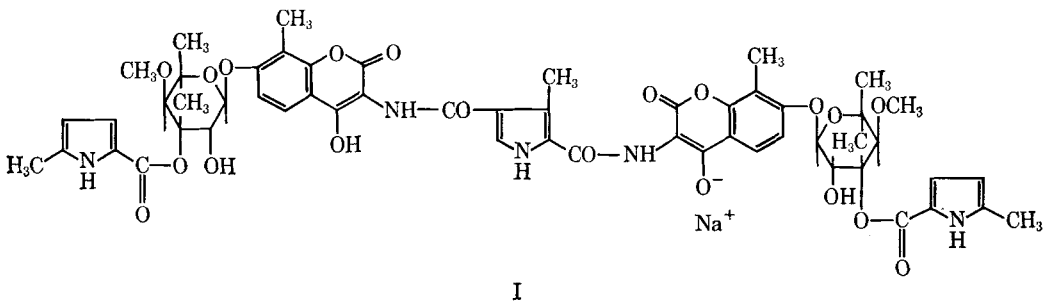
Abstract □ The pharmacokinetic profile of coumermycin A_1 has been determined in man following intravenous and oral administration. The antibiotic is eliminated slowly from the bloodstream and appears to be highly biotransformed. The plasma level *versus* time curve after intravenous injection is consistent with a two-compartment open system containing a primary compartment with a volume equivalent to the volume of plasma water. The design of a pharmacokinetic model is discussed.

Keyphrases □ Coumermycin A_1—pharmacokinetic profile □ Absorption, elimination—coumermycin A_1 □ Model, two-compartment open—coumermycin A_1 □ Kinetic equations—coumermycin A_1 absorption, elimination

Coumermycin A_1 is an antibiotic isolated from *Streptomyces hazeliensis* var. *hazeliensis* nov. sp. which exhibits antistaphylococcal activity *in vitro* and *in vivo* (1). The compound, a monosodium salt, has a molecular weight of 1132. Coumermycin A_1 is a bishydroxy coumarin with two weakly acidic groups which are widely separated spatially in the molecule and therefore ionize simultaneously with an approximate pKa of 6. The three pyrrole groups are weakly acidic, pKa > 11, and may decrease the solubility of the compound due to hydrogen bonding. The compound is only very slightly soluble in water at 25°. The structure is given as I (2):



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The pharmacokinetic profile of coumermycin A_1 was determined in four human subjects based on the serum level data obtained from the report of a clinical study on file (3, 4).

EXPERIMENTAL

Protocol—Four healthy human adults fasted overnight, received single intravenous and oral doses of coumermycin A_1 at the Special Treatment Unit of Martland Hospital. Subjects A and B each received single 50-mg. doses intravenously and orally, 3 weeks apart. Subjects C and D each received single 100-mg. doses intravenously and orally, 2 weeks apart. The drug was administered in the dosage forms presently used in clinical trials. Blood and urine specimens were collected periodically and the serum separated and frozen for subsequent analysis.

Microbiological Assay—Coumermycin A_1 was analyzed by the cup plate assay employing *Staphylococcus aureus* HLR No. 82. The sensitivity of the method is 0.08 mcg./ml. of biological fluid (4).

RESULTS AND DISCUSSION

The serum level data following both intravenous and oral administration of the drug are presented in Figs. 1–4. Following the intravenous injection of coumermycin A_1, a biexponential serum level curve is obtained with all four subjects. The consideration of the disposition of coumermycin A_1 in terms of a two-compartment open system model is therefore a minimal requirement in order to describe adequately the distribution of the drug into the body. The