Relative Bioavailability of Chlorthalidone in Humans: Adverse Influence of Polyethylene Glycol

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Abstract □ The bioavailability of two commercial preparations of chlorthalidone was studied in healthy male subjects. Reference solutions/suspensions for the two products were chlorthalidone dissolved in a solution of water–polyethylene glycol and a solution/suspension of chlorthalidone. Bioavailability of the chlorthalidone in water–polyethylene glycol solution was significantly reduced in comparison to one of the commercial preparations, and trends in the data suggested that it was less well absorbed than either the chlorthalidone in water solution/suspension or the other commercial preparation of chlorthalidone. These data, together with previous reports indicating that polyethylene glycol may retard the absorption of some drugs *in vitro*, suggest that this compound should not be used to aid dissolution of drug in a reference standard for bioavailability investigations.

Keyphrases □ Chlorthalidone—relative bioavailability in humans, adverse influence of polyethylene glycol □ Bioavailability—chlorthalidone in humans, adverse influence of polyethylene glycol □ Polyethylene glycol—adverse influence on relative bioavailability of chlorthalidone, humans

Chlorthalidone[1] [2-chloro-5-(1-hydroxy-3-oxo-1-isoindolinyl)benzene-sulfonamide] is a diuretic agent with a relatively long half-life (~45 hr), correspondingly long duration of effect, and a low incidence of adverse effects. Because it is now possible for several manufacturers to market the drug in the United States, studies will be performed to assess the bioequivalence of different formulations of chlorthalidone. Guidelines issued by the U.S. Food and Drug Administration (FDA) for the performance of these investigations recommend that bioequivalency studies of chlorthalidone be performed against a standard reference solution of chlorthalidone in at least 20 individuals in a two-way crossover design (1).

Because chlorthalidone is poorly soluble in water (12 mg/100 ml water at 20°) (2), FDA guidelines recommend a solution of chlorthalidone in polyethylene glycol (I) as the reference standard for bioavailability because polyethylene glycol aids the dissolution of the drug in water. Preliminary studies, however, have suggested that chlorthalidone in a water–polyethylene glycol solution is less well absorbed than a tablet formulation. To assess this possibility and to evaluate a generic preparation of chlorthalidone in accordance with FDA guidelines, a crossover investigation was designed to determine the relative bioavailability of different formulations of chlorthalidone.

EXPERIMENTAL

Clinical Study—The study was performed in the clinical facilities of the Drug Studies Unit, University of California, San Francisco. Subjects were healthy male volunteers between the ages of 21 and 40 and within 10% of standard weight for height and body frame size (3). Information about the study was given to each participant prior to entry and each subject signed a consent form indicating they were informed of the purpose and procedures of the study. The study lasted 12 weeks, during which the participants were administered a single dose of each of four preparations of chlorthalidone with a 3-week wash-out period between each dose.

Table I—Measurement of Blood Concentration–Time Curves in 12 Healthy Male Subjects After a Single 50-mg Dose of Chlorthalidone (Treatment A)

Parameter	Mean ± *SD*
T_{lag}, hr	0.513 ± 0.564
K_{abs}, hr^{-1}	0.168 ± 0.125
$T_{1/2_{abs}}$, hr	7.73
α, hr^{-1}	0.992 ± 0.623
$T_{1/2_\alpha}$, hr	0.929 ± 0.442
β, hr^{-1}	0.0181 ± 0.0082
$T_{1/2_\beta}$, hr	46.7 ± 22.2
V_1/F^a, l	14.4 ± 25.5
V_{dss}/F^a, l	11.0 ± 4.66
Vd_{area}/F^b	11.3 ± 4.05
Cl/F^a, liter/hr	0.184 ± 0.044
Cl/F^b, liter/hr	0.180 ± 0.046
Cl_{renal}, liter/hr	0.080 ± 0.018

[a] Calculated using computer fitted parameters. [b] Calculated using measured *AUC* values.

The following four formulations of chlorthalidone were administered as a single oral dose in the study: Two 25-mg chlorthalidone tablets[2] taken with 180 ml of water (Treatment A); chlorthalidone[3], two 25-mg tablets taken with 180 ml of water (Treatment B); chlorthalidone in water–polyethylene glycol solution, 50 mg/100 ml followed by an 80-ml water rinse of the dosing container (Treatment C); and chlorthalidone in solution/suspension, 50 mg/100 ml water, followed by an 80-ml water rinse of the dosing container (Treatment D). Chlorthalidone in solution or solution/suspension was prepared according to the following procedures. For treatment C, 10% water–polyethylene glycol 4000 solution was heated to 37° and added to chlorthalidone powder to give, after filtration, 50 mg of drug/100 ml of solution. Assay of solution, using the same assay as for blood, for Treatment C confirmed that the content of chlorthalidone was appropriate. For Treatment D, 50 mg of chlorthalidone was stirred in 100 ml of water prior to dosing.

Dose Administration—Twenty-two healthy males participated in the study. Twelve of the 22 subjects received all four formulations of chlorthalidone according to a balanced 4 × 4 Latin square design. Three subjects were assigned to each of four treatment sequences. An additional 10 subjects received only the formulation of chlorthalidone used in Treatment B and chlorthalidone in water–polyethylene glycol solution. Five of these subjects were randomly assigned to one of the two possible treatment sequences (product used in Treatment B followed by water–polyethylene glycol solution of drug) and five were assigned to the reverse procedure. Postexperiment analysis of the data indicated no sequence, period, or crossover effects in the 12 individuals receiving all four treatments. The data for Treatment B and for chlorthalidone in water–polyethylene glycol solution (Treatment C) from these 12 individuals were, therefore, combined with the data in the additional 10 subjects receiving these treatments to give 22 individuals who received both Treatments B and C. These data provided bioequivalency data for chlorthalidone tablets required by FDA for premarketing approval.

Dosing of chlorthalidone occurred at 8 am on a treatment day, followed by blood and urine collections for a period of 120 hr. Beginning with the 8 am dose, blood was collected at the following time periods: 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, and 120 hr. Urine was collected at the following time periods, beginning with the 8 am dose: 0–2, 2–4, 4–6, 6–8, 8–12,

[1] The drug is marketed as Hygroton by Ciba-Geigy in Europe and under license by USV Pharmaceuticals in the United States.

[2] U.S.V. Laboratories, lot No. 06009.

[3] Mylan Pharmaceuticals, lot No. E021M.

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Table II—Variables Assessing Bioequivalence Between Four Formulations of Chlorthalidone in 12 Subjects

Variable	Treatment[a] A	Treatment[b] B	Treatment[c] C	Treatment[d] D	P^e	Newman-Keuls: B versus A	Newman-Keuls: B versus C	Newman-Keuls: C versus D
$AUC_{0-\infty}$, mg × hr/liter	293 ± 67[f]	336 ± 53	278 ± 71	305 ± 74	0.074			
Amount in $urine_{0-120\ hr}$, mg	18.3 ± 2.5	22.1 ± 5.6	16.6 ± 3.4	17.9 ± 3.2	0.019	0.05	0.05	0.05
Ka, hr^{-1}	0.168 ± 0.13	0.253 ± 0.17	0.162 ± 0.13	0.185 ± 0.10	0.420			
β, hr^{-1}	0.0181 ± 0.0082	0.020 ± 0.010	0.0173 ± 0.004	0.0156 ± 0.0052	0.520			
C_{peak}, mg/l	3.73 ± 0.93	4.62 ± 0.80	3.36 ± 0.74	3.93 ± 1.12	0.022		0.05	
T_{peak}, hr	13.8 ± 6.3	10.8 ± 5.0	12.1 ± 11.7	16.1 ± 12.3	0.517			

[a] Tablet[2]. [b] Tablet[3]. [c] Chlorthalidone in water and polyethylene glycol. [d] Chlorthalidone solution/suspension in water. [e] Analysis of variance. [f] Standard Deviation.

12–24, 24–48, 48–72, 72–96, and 96–120 hr. Aliquots of urine were taken after volume measurement and both blood and urine samples were stored at −20° until analysis. Subjects fasted overnight (10 hr) prior to dosing and continued fasting until a standard lunch was administered 4 hr after dosing. They were ambulatory during the study but did not engage in strenuous exertion.

Drug Analysis—Chlorthalidone concentrations in whole blood and urine were assayed with high-pressure liquid chromatography (HPLC). Whole blood assay was performed because chlorthalidone is known to concentrate in red blood cells, whereas the concentrations in plasma are low. The method of assay is summarized here; however, details of the method will be presented in a separate publication.

Whole blood (0.2 ml) was mixed with an equal volume of distilled water, sonicated for 5 min, and mixed with acetonitrile containing phentolamine hydrochloride (internal standard). After mixing and centrifugation, the supernate was transferred to a clean tube and evaporated under nitrogen until ~0.4 ml of the solution remained. A portion of this sample was injected into the loop injector of the high-pressure liquid chromatograph. Urine was handled as blood with the exceptions that the sonication and centrifugation steps were not included and the urine internal standard was pentobarbital sodium. Chromatography was performed on a high-performance liquid chromatograph[4] equipped with a variable wavelength spectrophotometric detector[5]. The UV detector was set at 210 nm for blood and 250 nm for urine samples. The lower limit of sensitivity of the assay in blood was 200 ng/ml with a coefficient of variation of 5.0% and 750 ng/ml in urine with a coefficient of variation of 6.6%.

Pharmacokinetic and Statistical Analysis—To obtain the absorption and elimination rate constants, the blood concentration–time data were fitted to a two-compartment body model with first-order input and lag time. Due to the prolonged peak time, the absorption half-time was assumed to be longer than that for the fast disposition constant, α. This is consistent with values obtained previously (4) following intravenous dosing. Weighting of each data point was performed by squaring the value of the reciprocal of the observation. Parameter estimates were obtained using a nonlinear least-square computer program (3). The area under the plasma concentration curve (AUC) for chlorthalidone in blood was calculated by trapezoidal rule to C_{peak} and log-trapezoidal rule thereafter. $AUC_{120\ hr-\infty}$ was estimated from the concentration of chlorthalidone in blood at 120 hr divided by the terminal rate constant of elimination obtained from the fitting procedure. This area was added to the $AUC_{0-120\ hr}$ to give $AUC_{0-\infty}$. C_{peak} and T_{peak} represent the points of the highest concentration of chlorthalidone observed in blood and the time this concentration occurred. As verification of the fitting procedure, chlorthalidone clearance/F was also calculated using the noncompartmental method of dividing dose by $AUC_{0-\infty}$, where F is the fraction of the oral dose available to the systemic circulation. Renal clearance was determined by dividing the amount of drug excreted into the urine at 120 hr by the $AUC_{0-120\ hr}$.

The following parameters were analyzed statistically: absorption and elimination rate constants, $AUC_{0-\infty}$, amount of drug excreted in the urine in 120 hr, and C_{peak} and T_{peak} (5). Significant differences between treatments were identified using the Newman-Keuls test.

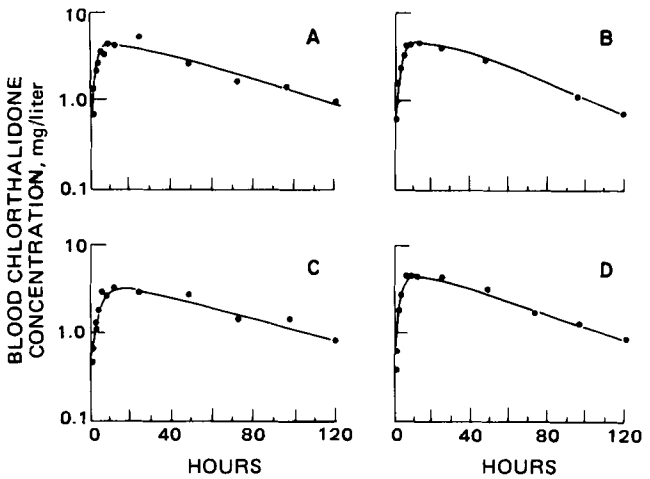

Figure 1—*Blood chlorthalidone concentration–time curves in a single individual (Subject 1) following administration of Treatment A, Treatment B, chlorthalidone in water/polyethylene glycol solution (Treatment C), and chlorthalidone in water solution/suspension (Treatment D).*

[4] Perkin-Elmer Series 3.
[5] Schoeffel, model SP 770.

RESULTS AND DISCUSSION

Representative blood concentration–time curves observed in a single individual (Subject 1) after each of the four treatments are shown in Fig. 1. Pharmacokinetic parameters derived from the curves in the 12 individuals receiving Treatment A are shown in Table I. Variables assessing bioequivalence in the 12 subjects who received all four treatments are shown in Table II and in the 22 subjects who received Treatments B and C in Table III. Table II also contains the results of the Newman-Keuls analysis that tested which of the four treatments differed. Table IV shows the comparison of values in all four preparations for the amount of drug excreted in the urine in 120 hr and the $AUC_{0-\infty}$. Because urine was collected for only approximately three half-lives of chlorthalidone, this measurement is probably less valuable in assessing chlorthalidone bioavailability than $AUC_{0-\infty}$.

The data in Table I correspond well with previous reports of chlorthalidone pharmacokinetics following an oral dose of the drug. Fleuren *et al.*, (4) reported a plasma clearance/F of 9.55 liter/hr for chlorthalidone (Treatment A) after a single dose of the drug. Using the Reiss *et al.* (6) value of 0.0138 for the plasma to blood ratio of chlorthalidone, this plasma clearance may be converted to a blood clearance of 0.132 liter/hr. This corresponds to the value of 0.184 liter/hr observed in this study for Treatment A, given the apparent variability in plasma to blood ratio of chlorthalidone over concentrations exhibited following clinical doses of the drug. This variability occurs as a consequence of saturable binding

Table III—Variables Assessing Bioequivalence Between Two Formulations of Chlorthalidone in 22 Subjects

Variable	Treatment B[a], mean ± SD	Treatment C[b], mean ± SD	P^c
$AUC_{0-\infty}$, mg/liter·hr	322 ± 60	272 ± 56	0.0023
Amount in $urine_{0-120\ hr}$, mg	20.2 ± 5.1	15.7 ± 3.3	0.0003
Ka, hr^{-1}	0.248 ± 0.20	0.134 ± 0.12	0.0315
β, hr^{-1}	0.0193 ± 0.0085	0.0168 ± 0.0045	0.2583
C_{peak}, mg/liter	4.03 ± 0.98	3.03 ± 0.69	0.0001
T_{peak}, hr	13.3 ± 8.9	18.2 ± 17.2	0.2134

[a] Tablet[3]. [b] Chlorthalidone in water and polyethylene glycol solution. [c] Analysis of variance.

Table IV—Comparison of $AUC_{0-\infty}$ and $Ae_{0-120\ hr}$ Values[a]: Ratio of Individual Values with Mean ± *SD*

Subject	$AUC_{0-\infty}$ Treatment A/C	$AUC_{0-\infty}$ Treatment B/C	$AUC_{0-\infty}$ Treatment D/C	$AUC_{0-\infty}$ Treatment B/A	Amount of Drug in $Urine_{0-120\ hr}$ Treatment A/C	Amount of Drug in $Urine_{0-120\ hr}$ Treatment B/C	Amount of Drug in $Urine_{0-120\ hr}$ Treatment D/C	Amount of Drug in $Urine_{0-120\ hr}$ Treatment B/A
1	0.97	1.23	1.00	1.26	1.45	1.43	1.43	0.98
2	1.04	0.94	0.93	0.90	0.74	1.56	1.01	2.11
3	1.56	1.09	1.53	0.70	1.37	1.49	1.61	1.08
4	1.51	1.57	1.18	1.04	1.19	1.42	1.48	1.19
5	0.89	0.79	1.13	0.89	1.67	2.23	1.96	1.34
6	1.46	0.97	1.62	0.66	1.30	1.07	1.32	0.83
7	1.16	1.36	1.05	1.17	0.86	1.08	0.84	1.24
8	1.27	1.60	1.08	1.25	0.80	0.90	0.82	1.12
9	1.11	1.43	0.86	1.28	1.49	1.55	1.32	1.04
10	0.97	1.87	1.23	1.92	1.28	1.14	1.68	0.90
11	0.97	1.10	1.06	1.13	1.41	1.71	1.22	1.22
12	1.31	1.32	0.99	1.01	1.04	0.83	0.63	0.80
13		0.95				1.02		
14		1.38				0.93		
15		1.13				1.23		
16		1.18				1.67		
17		1.09				1.32		
18		0.94				1.18		
19		1.40				1.34		
20		1.02				1.02		
21		0.88				1.24		
22		1.66				1.37		
Mean	1.19	1.22	1.14	1.10	1.22	1.31	1.28	1.15
SD	0.23	0.28	0.23	0.33	0.30	0.32	0.39	0.34

[a] Treatments A, B, and D compared with Treatment C; Treatment B compared with A.

of drug to erythrocytes (7). Fleuren *et al.* (4) reported a terminal half-life of 44.1 hr, whereas a value of 46.7 hr was obtained in this study. Although absolute bioavailability could not be assessed in this study, Fleuren and coworkers reported that the fraction of drug absorbed into the systemic circulation after an oral dose is ~0.60. Multiplying the clearance/*F* values by 0.60 yields a blood chlorthalidone clearance of ~0.110 liter/hr (1.84 ml/min). Slightly less than three-quarters of the chlorthalidone clearance is represented by renal elimination of unchanged drug, while the remainder presumably occurs as a result of hepatic biotransformation and biliary excretion. With a low distribution volume and a relatively long terminal half-life, chlorthalidone belongs to the category of drugs that are poorly extracted from the blood.

The data in Table III demonstrate a statistically significant difference between Treatments B and C for several parameters, apparently because of the larger number of individuals studied. From this study, Treatment B is absorbed more rapidly, attains a higher concentration of chlorthalidone in the blood, produces a greater $AUC_{0-\infty}$, and results in a greater amount of drug excreted in the urine by 120 hr in comparison with the drug in the water–polyethylene glycol solution. The data in this table also indicate that for $AUC_{0-\infty}$, Treatment B differs from Treatment C (as does Treatment A) according to the 75:75 rule (FDA), in which 75% of the individuals must demonstrate a test value within 75% of the value for the reference solution.

Previous investigations have suggested that polyethylene glycol can retard drug absorption. In 1966 it was demonstrated in two different *in vitro* systems that polyethylene glycol retarded the dissolution and absorption of phenobarbital (8). It was suggested that this effect probably occurred as a consequence of complexation of phenobarbital with polyethylene glycol. This particular phenomenon was observed only for phenobarbital, however, and not for pentobarbital, barbital, or barbituric acid. It was demonstrated more recently using the *in situ* rat gut technique, that both polyethylene glycol 4000 and 6000 retarded the rate of disappearance of salicylic acid from the gut (9). The clinical data in this report corroborate these *in vitro* observations on the potential influence of polyethylene glycol on drug dissolution and/or absorption.

Polyethylene glycol 4000 is one of several polyethylene glycols produced by reacting ethylene oxide with ethylene glycol or water; they bear the general formula of $H(CH_2CH_2)_nOH$. Used as demulcents, polyethylene glycols are found in water-soluble ointment bases, as ingredients of lotions and suppositories, and as tablet coatings. Although polyethylene glycol 4000 results in improved solubility of chlorthalidone in water, the data in this investigation, as well as previous reports (8, 9), indicate that it is not an appropriate constituent for use in chlorthalidone bioavailability studies. Its use in bioequivalency investigations for other drugs should probably be discouraged until it can be shown to be inert in the test system employed.

The data in Tables II and IV suggest also that Treatment B may be slightly more bioavailable than a water solution/suspension of chlorthalidone or the currently marketed preparation of the drug, Treatment A. Whatever differences exist between the treatments in this study, however, are not likely to be of clinical significance. These observations raise the following issue: As pharmaceutical technology advances, generic formulations of marketed drugs may be produced that are substantially more bioavailable than the innovators' products. In such circumstances, the FDA may be in the unenviable position of requesting that generic manufacturers either design a product with lower bioavailability characteristics or alter the drug content of their formulation. As an alternative, the FDA may ask the innovator to reformulate. Whatever the final decision, the results of this study emphasize the importance of including the innovator's formulation in assessing the bioequivalence of a generic product.

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