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COMPARATIVE BIOAVAILABILITY AND PHARMACOKINETICS OF HYDROCHLOROTHIAZIDE FROM ORAL TABLET DOSAGE FORMS, DETERMINED BY PLASMA LEVEL AND URINARY EXCRETION METHODS

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ABSTRACT

The bioavailability and pharmacokinetics of two hydrochlorothiazide products were compared following single 50 mg oral doses to 20 healthy male volunteers. Plasma and urine were assayed for hydrochlorothiazide by a specific and sensitive HPLC method. Plasma profiles of hydrochlorothiazide were adequately described by a triexponential function. The bioavailability of hydrochlorothiazide from the two brands did not differ significantly as judged by the values of C_{max}, t_{max}, $AUC^{0\rightarrow\infty}$, mean residence time, variance of residence time, and urinary excretion of unchanged drug. Close similarity was observed between urinary excretion rates and concentrations of drug in plasma.

KEY WORDS Hydrochlorothiazide Bioavailability Pharmacokinetics Plasma levels Urinary recovery

INTRODUCTION

The majority of studies concerning hydrochlorothiazide bioavailability have been based on urinary excretion measurements.[1–3] In studies in which plasma levels of drug were also obtained, poor agreement was reported between results obtained from the two fluids.[4–6,7] Spectrophotometric assays employed for the determination of hydrochlorothiazide in urine, for bioavailability estimations, are non-specific because of interference by endogenous urinary constituents.[8]

Recent studies in this laboratory has confirmed that areas under hydrochlorothiazide plasma curves do not correlate well with urinary excretion data

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among individuals.[7] However, good agreement was obtained between mean values derived from different drug treatments, and close similarity was observed between urinary excretion rates of hydrochlorothiazide and the time course of drug concentrations in plasma.[7]

The purpose of this investigation was to examine the relative bioavailability of two oral tablet dosage forms of hydrochlorothiazide, and to compare plasma level and urinary excretion data, using a specific and sensitive high pressure liquid chromatography (HPLC) assay,[9] for bioequivalency estimation.

MATERIALS AND METHODS

Subjects

Twenty healthy male subjects, aged 22–46 years (mean 28) and weighing 65–107 kg (mean 75), participated in the study after passing a physical examination and giving informed consent. Subjects were not permitted to take any other medication for 2 weeks before or during the study. Ingestion of tea, coffee, carbonated, and alcoholic beverages was prohibited during the entire plasma and urine sampling period.

Protocol

Tablets were Hydrodiuril© hydrochlorothiazide 50 mg tablets Lot B0686, Merck Sharpe and Dohme (MSD), West Point, PA 19486, and Hydrochlorothiazide 50 mg tablets, Lot 177903, Stanlabs, Portland, OR 97214.

Twenty subjects were randomly divided into 2 groups of 10. One group received 1 tablet of the MSD formulation first, followed by 1 tablet of the Stanlab formulation 1 week later, while the other group received the tablets in reverse order.

Subjects were instructed to ingest no solid food after 8 p.m., and no liquid after 10 p.m. on the day preceding a study day. On the morning of a drug treatment, subjects drank 240 ml of water on arising, between 6.30 and 7.00 a.m. A single 50 mg hydrochlorothiazide tablet was administered together with 240 ml of water at 8 a.m. No food was permitted on a treatment day until 4 h after dosing, when a light lunch was provided. Subjects drank 120 ml of water at the end of each postdose urine collection interval.

Blood samples ($\sim$10 ml) were drawn from a forearm vein into Vacutainers containing heparin as anticoagulant immediately before and then at 0·5, 1, 1·5, 2, 4, 6, 8, 12, and 24 h after dosing. Plasma was separated and stored at $-20°$ until assayed. Urine was collected immediately before and then quantitatively during 0–1, 1–2, 2–3, 3–4, 4–6, 6–8, 8–12, 12–24, and 24–48 h intervals after dosing. Urine volumes and pH values were recorded and a 25 ml sample was stored at $-20°$ until assayed.

Assay

Concentrations of hydrochlorothiazide in plasma and urine were determined by the HPLC methods reported previously.[9] The methods are linearly sensitive to drug concentrations between 2 and 100 $\mu g\,ml^{-1}$ in urine and between 10 and 750 $ng\,ml^{-1}$ in plasma. Coefficients of variation from multiple determinations within these concentration ranges were within 10 per cent of the mean.

Analysis of the data

Pharmacokinetic analysis. Individual plasma hydrochlorothiazide concentrations were fitted to a triexponential function, as in equation (1), where

$$C = Xe^{-\alpha t} + Ye^{-\beta t} + Ze^{-\gamma t} \tag{1}$$

where C is the drug concentration at time t; X, Y, and Z are concentration terms, and α, β, and γ are first-order rate constants. Initial estimates of parameter values were obtained by standard graphical methods. Improved estimates and statistical analysis were obtained by non-linear regression using the computer program NREG.[10] Areas under plasma hydrochlorothiazide concentration versus time curves from zero to infinite time, AUC, were calculated by trapezoidal rule with end correction where necessary. Other pharmacokinetic parameters used to determine the rate and extent of hydrochlorothiazide bioavailability from the two formulations were the peak drug concentrations in plasma, C_{max}, the time, t_{max}, at which C_{max} occurred, and the cumulative 0–48 h urinary recovery of hydrochlorothiazide, A_e.

The relative efficiency of hydrochlorothiazide absorption from the Stanlab and MSD formulations (F_S/F_M) was estimated by four different methods:

Method 1 assumes that plasma clearance of hydrochlorothiazide is constant and is based on equation (2), where D is the oral dose and the subscripts S and M refer to Stanlab and MSD formulations, respectively.

$$\frac{F_S}{F_M} = \frac{D_M(AUC)_S}{D_S(AUC)_M} \tag{2}$$

Method 2 assumes that the non-renal component of hydrochlorothiazide plasma clearance remains constant, but a correction is incorporated for changing renal clearance, as in equation (3),[11]

$$\frac{F_S}{F_M} = \frac{D_M(AUC)_S}{D_S(AUC)_M} - (Cl_{rM} - Cl_{rS})\frac{(AUC)_S}{D_S} \tag{3}$$

where Cl_r is the renal clearance of hydrochlorothiazide and is calculated from equation (4), where A_e is

$$Cl_r = \frac{A_e}{AUC} \tag{4}$$

the quantity of drug recovered unchanged in 48 h urine.

Method 3 assumes that any change in renal clearance is accompanied by a proportional change in non-renal clearance, as in equation (5).

$$\frac{F_S}{F_M} = \frac{(D_M)(Cl_r)_S(AUC)_S}{(D_S)(Cl_r)_M(AUC)_M} \tag{5}$$

Method 4 is based on the dose-corrected amounts of drug excreted in urine, as in equation (6).

$$\frac{F_S}{F_M} = \frac{(A_e)_M(D_S)}{(A_e)_S(D_M)} \tag{6}$$

Statistical moments analysis of plasma data. In addition to the above methods, plasma data were also analysed by statistical moments analysis in terms of mean residence time (MRT) and variance of residence time (VRT).[12] These values, which are respectively the first normal and second central moments of the plasma drug profile, are defined by equations (7) and (8).

$$\text{MRT} = \int_0^\infty t \cdot C \cdot dt/\text{AUC} \tag{7}$$

$$\text{VRT} = \int_0^\infty (t - \text{MRT})^2 \cdot C \cdot dt/\text{AUC} \tag{8}$$

The MRT and VRT have advantages over other methods of calculating drug bioavailability in that they reflect not only the extent of absorption (AUC) but also the rate of transit (resident time) of drug molecules through the body. They are also pharmacokinetic model independent.[12]

The ratios MRT_S/MRT_M and VRT_S/VRT_M, where the subscripts are as defined previously, provide a measure of the extent of absorption from the two tablet formulations.

Statistical analysis. Plasma and urine hydrochlorothiazide concentrations at each sampling time and all derived pharmacokinetic parameter values were examined for treatment, group, and treatment–group interaction effects by analysis of variance for crossover design.

RESULTS AND DISCUSSION

Mean plasma hydrochlorothiazide concentrations in the 20 individuals are shown, together with standard deviations and coefficients of variation, in Table 1. From both dosages peak mean concentrations of *c.* 290 ng ml^{-1} were obtained at 2 h postdosing. Drug levels then declined rapidly until 8–12 h, and subsequently at a slower rate.

Visual inspection of individual plasma profiles revealed the same triphasic pattern reported previously.[7,9] Plasma data were therefore fitted by means of equation (1), yielding the numerical values ($\pm$ S.D.) shown in equations (9) and (10).

Table 1. Mean plasma hydrochlorothiazide concentrations

Formulation	Plasma hydrochlorothiazide (ng ml^{-1}) at (h) 0·5	1	1·5	2	4	6	8	12	24
MSD	53	197	261	287	221	123	84	51	22
±S.D.*	52	108	123	104	67	39	20	13	6
CV%	98	55	47	36	27	23	21	26	27
Stanlab	47	190	273	295	238	136	91	54	23
±S.D.	49	141	119	108	75	41	24	13	6
CV%	104	74	43	37	31	29	26	24	26

* Standard deviation.

MSD:

$$C = 967 \pm 997\, e^{-0.34 \pm 0.06t} + 125 + 43\, e^{-0.072 \pm 0.02t} - 1126 \pm 1016\, e^{-0.85 \pm 0.33t} \quad (9)$$

Stanlab:

$$C = 1121 \pm 845\, e^{-0.35 \pm 0.09t} + 135 \pm 43\, e^{-0.075 \pm 0.02t} - 1297 \pm 840\, e^{-0.79 \pm 0.39t} \quad (10)$$

The mean coefficients of determination, $r^2 = ((\Sigma obs^2 - \Sigma dev^2)/\Sigma obs^2)$, from non-linear regression analysis were $0{\cdot}946 + 0{\cdot}03$ and $0{\cdot}932 \pm 0{\cdot}004$ from the MSD and Stanlab formulations, respectively.

Mean cumulative urinary excretion of hydrochlorothiazide during 48 h postdosing is shown in Table 2. Urinary recovery accounted for *c.* 75 per cent of both formulations. Urine pH values were within the normal range throughout.

Table 2. Mean cumulative percentage of administered hydrochlorothazide recovered in urine

Formulation	Percentage recovered at (h) 0–1	1–2	2–3	3–4	4–6	6–8	8–12	12–24	24–48
MSD	3·2	13·5	24·1	33·8	43·5	50·3	57·5	67·5	74·4
±S.D.	3·5	5·3	6·1	8·6	8·3	8·5	10·1	9·7	10·2
CV%	109·3	39·3	25·3	25·4	19·1	19·5	17·5	14·4	13·7
Stanlab	2·9	13·8	24·6	34·4	45·3	52·5	60·1	70·7	76·8
±S.D.	3·0	6·6	7·4	7·1	7·4	7·9	8·9	9·6	9·5
CV%	103·4	47·8	30·1	20·6	16·3	15·0	14·8	13·6	12·4

Analysis of variance showed no significant difference in plasma levels at each sampling time, cumulative urinary excretion, or urinary excretion rates of hydrochlorothiazide due to group, treatment, or group–treatment interactions from the two tablets. As observed previously, urinary excretion rates of hydrochlorothiazide correlated well with individual plasma levels, exhibiting a triphasic pattern.[7,9]

The bioavailability and pharmacokinetic parameters obtained from analysis of plasma and urine data are summarized in Table 3. The values obtained are similar to those reported previously,[7] and there were no significant differences in any value between the two tablet formulations. Thus, plasma profiles of

Table 3. Summary of mean bioavailability and pharmacokinetic parameter values (±1 S.D.) from plasma and urine data, and results of statistical analysis

Parameter	Value		Statistic
	MSD	Stanlab	
C_{max}* ($ng\,ml^{-1}$)	310±115	331±103	NSD†
t_{max}‡ (h)	2·1±0·9	2·5±1·1	NSD
AUC ($ng\,h\,ml^{-1}$)	2442±676	2583±580	NSD
$\Delta u/\Delta t_{max}$§ ($mg\,h^{-1}$)	7·1±3·0	6·6±1·5	NSD
β‖ (h^{-1})	0·072±0·020	0·075±0·020	NSD
$t_{\frac{1}{2}(\beta)}$¶ (h)	9·6±2·7	9·3±2·5	NSD
MRT** (h)	12·4±3·2	11·8±2·1	NSD
VRT†† (h^2)	212·7±130·6	184·3±87·1	NSD
Cl_r‡‡ ($ml\,min^{-1}$)	289±92	275±67	NSD
A_e§§	74·4±10·2	76·8±9·5	NSD

* Maximum plasma concentration of hydrochlorothiazide.
† No significant differences by analysis of variance.
‡ Time of maximum drug levels in plasma.
§ Maximum urinary excretion rate.
‖ Smallest of the three rate constants obtained by analysis of plasma data according to equation (1).
¶ Terminal elimination half-life, calculated from $t_{\frac{1}{2}(\beta)} = 0{\cdot}693/\beta$.
** Mean residence time of hydrochlorothiazide in plasma. For explanation see text.
†† Variance of residence time in plasma. For explanation see text.
‡‡ Renal clearance, calculated from equation (4).
§§ Percentage of administered dose recovered in 0–48 h urine.

hydrochlorothiazide from 50 mg oral tablet doses are characterized by peak levels of approximately $300\,ng\,ml^{-1}$ occurring at 2·1–2·5 h, and biphasic elimination with fast and slow component half-lives of approximately 2 and 9 h, respectively.

The relative absorption efficiency of hydrochlorothiazide, calculated by means of equations (2), (3), (5), and (6), and also ratios MRT_S/MRT_M and VRT_S/VRT_M, are given in Table 4. The F_S/F_M ratios calculated from equations (2), (3), (5), and (6) were not significantly different from each other, or from unity. The statistical moments analysis of plasma data also gave rise to F ratios close to unity. The mean ratio based on VRT values was somewhat low at 0·87±0·44, but the ratios from both statistical moments methods were not significantly different than those obtained by conventional methods.

As the ratio obtained from equation (6) is based on urinary excretion, while all other ratios are based on plasma levels of hydrochlorothiazide, we conclude

Table 4. Relative absorption efficiency (±1 S.D.) of hydrochlorothiazide from two tablet formulations, calculated from equations (2), (3), (5), and (6), and also from ratios of mean residence times and variance of residence time

Method of calculation (equation)	F_S/F_M
(2)	1·08±0·14
(3)	0·97±0·15
(5)	1·04±0·12
(6)	1·04±0·11
MRT_S/MRT_M	0·95±0·21
VRT_S/VRT_N	0·87±0·44

that for the formulations used in this investigation urine and plasma data yield equivalent estimates of relative bioavailability. In fact either one of the six methods used here, with the possible exception of VRT, might be expected to yield essentially identical results. Poor correlations between urinary excretion and areas under hydrochlorothiazide plasma curves that were reported previously,[4–6] appear to be due to variability in individual data, and also to relatively small treatment effects, rather than the absence of a true relationship. Support for this argument is provided by a previous study concerning drug–food interactions influencing the hydrochlorothiazide absorption.[7] Although urinary excretion correlated poorly with AUC values in that study, the correlations between the means of these values from different dosing treatments was high ($r = 0·996$).

The results obtained in the present study indicate that either plasma level or urinary excretion measurements are suitable to determine hydrochlorothiazide bioavailability, and also that a variety of methods yield equivalent results.

We recognize that a more stringent comparison of the methods described in this study would be obtained with drug products that have dissimilar absorption characteristics. However, the inability by Meyer *et al.*[2] to show bioavailability differences among 13 hydrochlorothiazide products, based on urine data, suggests that identifying such products may not be a simple task.

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