Chem., **193**, 265 (1951).
(20) R. L. Lester, "Methods in Enzymology," vol. 10, R. W. Estabrook and M. E. Pullman, Eds., Academic, New York, N.Y., 1967, p. 264.
(21) H. Lees, *Biochim. Biophys. Acta*, **105**, 184 (1965).
(22) J. B. Sumner, *Science*, **100**, 413 (1944).
(23) D. K. Myers and E. C. Slater, *Biochem. J.*, **67**, 558 (1957).
(24) C. H. Fiske and Y. Subbarow, *J. Biol. Chem.*, **66**, 375 (1925).
(25) R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep. Part 3*, **1972**, 3.

ACKNOWLEDGMENTS

The authors thank the Medical Research Council of Canada for the award of an operating grant (MA-5538) to J. R. Dimmock and a Summer Undergraduate Research Scholarship to E. W. K. Chow. Acknowledgment is made with gratitude to the University of Saskatchewan who provided a Graduate Scholarship to D. L. Kirkpatrick and also the University of Saskatchewan Alumni Association who awarded D. L. Kirkpatrick the John and Mary Spinks Scholarship.

Pharmacokinetic Interactions of Tolazamide and Oxyphenbutazone in Dogs

S. E. ABIDI *, A. H. KIBBE ˣ, R. W. CLEARY, A. B. JONES, and E. C. HARLAND

Received October 14, 1980, from the *Department of Pharmaceutics University of Mississippi, University, MS 38677.* Accepted for publication May 13, 1981. * Present address: School of Pharmacy, West Virginia University, Morgantown, WV 26506.

Abstract □ The described pharmacokinetic analysis involved two separate studies on nine dogs randomly assigned to three groups of three dogs each. In the first study, the effect of varying the dosage of tolazamide was examined. The second study concerned the effect of varying the dosage of oxyphenbutazone on tolazamide. A 3 × 3 Latin square was used to study both effects. Each group received each treatment, with a minimum of 1 week separating each session. The pharmacokinetics of tolazamide followed a two-compartment open model. The hybrid rate constants, α and β, were not significantly different at the three dosages when measured by a three-way analysis of variance. The only significant difference at the three dosage levels of tolazamide was in the apparent volume of distribution. In the pharmacokinetic interaction associated with intravenous administration of one dose of tolazamide and three doses of oxyphenbutazone, the apparent volume of distribution and the hybrid rate constant α did not change significantly while the hybrid rate constant for tolazamide, β, seemed to decrease with increasing oxyphenbutazone.

Keyphrases □ Pharmacokinetics—tolazamide and oxyphenbutazone interactions, dogs □ Tolazamide—pharmacokinetic interactions with oxyphenbutazone, dogs □ Oxyphenbutazone—pharmacokinetic interactions with tolazamide, dogs

Enhanced hypoglycemic effects have been reported when patients, stabilized on a sulfonylurea (tolbutamide or tolazamide), have had phenylbutazone and/or its analog, oxyphenbutazone, added to the treatment regimen (1). Hussar (2) suggested that phenylbutazone/oxyphenbutazone enhanced the hypoglycemic effects of tolbutamide or tolazamide.

Other investigators (3) found significant prolongation of the half-life of tolbutamide when phenylbutazone was administered at the same time. However, there have been few studies on this pharmacokinetic interaction and its clinical significance. None of the studies dealt with the pharmacokinetic parameters involved in the interaction of oxyphenbutazone and tolazamide when administered simultaneously.

There are three possible mechanisms for a pharmacokinetic interaction that enhances the hypoglycemic effects of tolazamide and other sulfonlyureas in the presence of phenylbutazone or oxyphenbutazone:

1. Inhibition of drug metabolism, *i.e.*, a decrease in metabolism of tolazamide/sulfonylurea, which could be caused by the presence of oxyphenbutazone or phenylbutazone.
2. Displacement of tolazamide/sulfonylurea from protein binding sites, which could result in increased blood concentrations of unbound, pharmacologically active tolazamide/sulfonylurea or its metabolites and could also produce high urinary concentrations of unchanged drug and its metabolites.
3. Inhibition of the excretion of tolazamide/sulfonylurea by a direct effect on the kidney.

The purpose of this study was to determine if a measurable pharmacokinetic interaction existed between tolazamide and oxyphenbutazone and which mechanism might explain it.

EXPERIMENTAL

Experimental Design—A 3 × 3 Latin square was used to study the effect of varying dosage on tolazamide pharmacokinetics. A similar Latin square design was used for the tolazamide–oxyphenbutazone interaction study, in which the dose of tolazamide was kept constant and the doses of oxyphenbutazone were varied.

Nine dogs were randomly assigned to three groups of three dogs each. Each group received each treatment, with a minimum of 1 week of rest separating each session.

The study was conducted over 3 months, with the sequence of treatments in accordance with a Latin square design (Table I). In the first study, Treatments A, B, and C were 5, 20, and 35 mg of tolazamide/kg, respectively. In the second study, Treatments A, B, and C were 10, 15, and 20 mg of oxyphenbutazone/kg, respectively, all with 20 mg of tolazamide/kg.

Typical Sampling Times—Zero time for tolazamide sampling was taken to be the midpoint of the tolazamide injection. Sampling times were 2, 5, 10, and 30 min and 1, 2, 4, 6, 8, 12, and 24 hr after the tolazamide injection.

Animals—Nine mongrel dogs, 9.0–16.5 kg, were obtained from the local animal shelter. These animals were vaccinated and given appropriate treatment for parasites; after acclimation, they were certified as healthy.

A catheter was surgically placed in the jugular vein so that the tip was

Table I—Latin Square Design for the Study of Tolazamide Pharmacokinetics

Group	Dog Identification Number	Week 1	2	3
1	1, 2, 3	A	B	C
2	4, 5, 6	B	C	A
3	7, 8, 9	C	A	B

0022-3549/82/0100-0029$01.00/0

Table II—Pharmacokinetic Parameters (Mean ± *SD*) Calculated from Tolazamide Plasma Concentrations Obtained after Intravenous Administration of Three Dosages

Parameter	Treatment[a] A	B	C
A, μg/ml	21.51 (4.15)	69.14 (35.36)	124.07 (53.38)
B, μg/ml	21.17 (8.69)	114.49 (37.91)	234.71 (42.24)
α, hr^{-1}	5.69 (2.40)	5.84 (4.63)	7.04 (3.37)
β, hr^{-1}	0.11 (0.01)	0.11 (0.02)	0.11 (0.02)
C_p^0, μg/ml	43.40 (11.43)	179.90 (61.82)	362.27 (64.91)
k_{21}, hr^{-1}	2.94 (1.44)	3.38 (1.99)	4.48 (2.50)
k_e, hr^{-1}	0.23 (0.04)	0.18 (0.08)	0.17 (0.04)
k_{12}, hr^{-1}	2.83 (2.07)	2.30 (2.16)	2.44 (1.68)
k_{12}/k_{12}	0.91 (0.38)	0.59 (0.47)	0.53 (0.28)
$V_d$ext liters	3.02 (0.80)	2.40 (0.96)	1.91 (0.50)

[a] A is 5, B is 20, and C is 35 mg of tolazamide/kg of body weight.

in the anterior vena cava. The catheter was directed subcutaneously from the jugular vein to a 2.0-cm incision in the back between the scapulae. It was attached to a 18-gauge needle with an infusion cap to allow for ease of sample removal.

Preparation of Material—The dogs were weighed the afternoon prior to treatment. The amount of the drug to be administered was determined according to the corresponding dose for that treatment session and the weight of the animal. Tolazamide and oxyphenbutazone solutions for intravenous injection were prepared and filtered through a 0.22-μm filter to ensure sterility.

Treatment—Food, but not water, was withdrawn from the dogs 12 hr prior to administration. Food was allowed *ad libitum* after the collection of the 4-hr sample. Dogs 1–9 received 5, 20, or 35 mg of tolazamide/kg of body weight iv in the first phase of the study.

In the second phase, Dogs 1–9 received intravenous injections of 20 mg of tolazamide/kg of body weight, immediately followed by 10, 15 or 20 mg of oxyphenbutazone/kg of body weight iv. Intravenous administration was selected to avoid any problem with drug absorption that would prevent determination of the volume of distribution.

The dose of tolazamide was selected to obtain measurable blood concentrations of the drug for at least three to five biological half-lives. This procedure allowed reproducible measurement of the magnitude of peak concentrations as well as accurate measurement of the lower values at the end of the experiment. Blood samples were withdrawn through a catheter or by venous puncture. A 3.0-ml blood sample was mixed with heparin and centrifuged immediately after mixing. The plasma was separated, transferred into a screw-capped culture tube (7 mm), and frozen at −20° until assayed.

Preparation and Extraction of Plasma Standards—A standard solution of tolazamide was prepared by dissolving 100 mg of tolazamide in 5 ml of 0.1 *N* NaOH in a 100-ml volumetric flask and brought to volume with distilled water.

Aliquots of the standard solution of tolazamide containing 5, 10, 20, 30, 40, 50, and 60 μg were placed in 50-ml screw-capped centrifuge tubes. To each centrifuge tube, 0.5 ml of dog plasma was added and mixed well. An aliquot (0.2-ml) of the internal standard solution containing 20 μg of trihexyphenidyl was then added to each centrifuge tube. To these samples, 1 ml of buffer solution (0.5 *M* KH_2PO_4,Na_2HPO_4) of pH 6 was added. Then 20 ml of chloroform was added to each centrifuge tube. The centrifuge tubes were shaken for 20 min on a table shaker and centrifuged for 5 min at 100 × *g* to break the emulsion. The aqueous phase was removed by aspiration, and 10 ml of the organic phase was transferred to a 10-ml sample vial. Samples were evaporated at room temperature in a hood overnight. The residue was dissolved in 20 μl of chloroform and analyzed by gas chromatography (GC).

Plasma Specimens—Aliquots (0.5 ml) of the plasma samples were placed in 50-ml centrifuge tubes, and the samples were processed in the same manner as the plasma standards. Duplicate aliquots from each sample were analyzed. Analysis was carried out on a gas chromatograph[1] equipped with a flame-ionization detector and a glass column (U-shaped, 183 cm length × 2-mm i.d.) packed with 3% OV-17 on 100–200 mesh Gas Chrom Q. Nitrogen was the carrier gas at a flow rate of 65 ml/min, and the rates for air and hydrogen were 250 and 45 ml/min, respectively. The column oven was operated isothermally at 180°, the flash heater was at 230, and the detector was at 280°. Under these conditions, tolazamide and trihexyphenidyl had retention times of 4 and 20 min, respectively.

[1] Beckman GC-45.

Table III—Statistical Analysis of $V_d$ext for Tolazamide at Three Dosages

Source of Variation	*df*	*SS*	*MS*	*F*
Total	26	19.86	—	—
Subject	8	8.71	1.09	2.86[b]
Group	2	0.78	0.39	1.03[a]
Subject/group	6	7.93	1.32	3.47[b]
Week	2	0.28	0.14	0.37[a]
Treatment	2	0.28	0.14	0.37[a]
Residual	14	5.36	0.36	—

[a] Not significant.
[b] Significant at the 1% level.

When using peak height ratios of tolazamide to internal standard, the calibration data (over concentration ratios of 0.25–3.00 and peak height ratios of 0.10–1.25) were linear with a correlation coefficient of 0.994.

Assay Specificity—To ensure assay specificity, a standard solution (1 mg/ml) of tolazamide was prepared in chloroform. Similarly, a standard solution of the internal standard (1 mg/ml) was prepared.

Five microliters of the standard solution of tolazamide were placed in a sample vial, and 2 μl were analyzed by GC. Retention times under the described conditions were 4 min for tolazamide and 20 min for trihexphenidyl.

As a further check on specificity, the following experiment was performed on one dog's plasma extract. Plasma extract (20 μl) was chromatographed on precoated TLC plates of silica gel F-254 of 250-μm thickness. A reference sample containing 20 μg of pure tolazamide was utilized. The plate was developed in a solvent system of chloroform–methanol–formic acid (59:4:1). The zones of tolazamide were located by examination of the chromatogram using a UV lamp (254 nm).

The R_f value (0.59) of tolazamide from all plasma extracts was identical to that of the pure tolazamide sample. The zones were removed separately and extracted with chloroform (3 × 2 ml). To each extract was added 0.1 ml of internal standard, and the total was evaporated to dryness. The residue was reconstituted in 15 μl of chloroform and analyzed. The chromatogram showed identical retention times for the TLC extract of pure tolazamide and for the samples obtained from separately extracted spots from dog plasma extracts.

Data Analysis—Results obtained from analysis of the plasma samples were corrected to reflect the plasma tolazamide concentration. These plasma concentrations and their corresponding times were utilized to calculate the pharmacokinetic parameters for each animal using AUTOAN (4).

Analysis of variance was performed to determine the significance of the differences between pharmacokinetic parameters due to treatment effect, variation among subjects and time period, and overall residual effect.

RESULTS AND DISCUSSION

The pharmacokinetic analysis involved two separate studies on an equal number of dogs. In the first study, the effect of varying the tola-

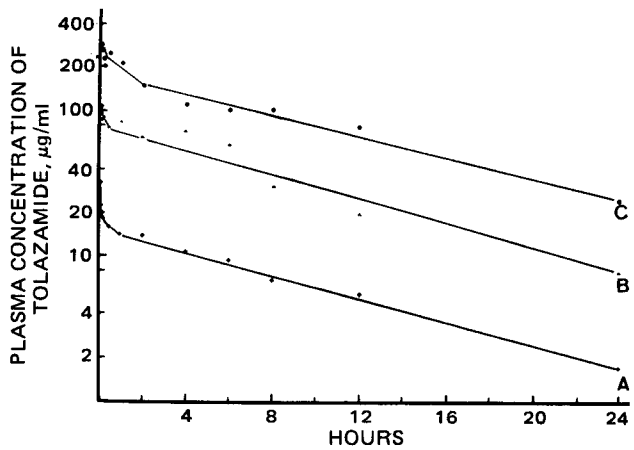

Figure 1—*Plasma concentration* versus *time curves of tolazamide for Dog 9. Key: curve A (+);5 mg of tolazamide/kg of body weight; curve B (△), 20 mg of tolazamide/kg of body weight; and curve C (●), 35 mg of tolazamide/kg of body weight.*

Table IV—Pharmacokinetic Parameters (Mean ± *SD*) Calculated from Tolazamide Plasma Concentrations Obtained after Intravenous Administration of Three Dosages of Oxyphenbutazone

Parameter	Treatment[a] TA	TB	TC
A, μg/ml	57.67 (23.29)	78.17 (35.71)	61.49 (42.18)
B, μg/ml	106.85 (20.47)	109.36 (17.68)	120.30 (36.74)
α, hr^{-1}	9.31 (7.18)	8.02 (5.64)	8.47 (6.07)
β, hr^{-1}	0.14 (0.02)	0.11 (0.02)	0.09 (0.02)
C_p^0, μg/ml	165.50 (31.69)	181.58 (32.63)	178.07 (41.92)
k_{21}, hr^{-1}	4.54 (3.71)	5.83 (4.87)	4.42 (2.96)
k_e, hr^{-1}	0.03 (0.16)	0.18 (0.05)	0.15 (0.04)
k_{12}, hr^{-1}	4.36 (3.89)	4.20 (4.56)	3.15 (1.84)
k_{12}/k_{12}	1.07 (1.09)	0.64 (0.25)	0.63 (0.35)
$V_d$ext, liters	2.31 (0.73)	2.21 (0.56)	2.23 (1.01)

[a] T is 20 mg of tolazamide/kg of body weight, and A is 10, B is 15, and C is 20 mg of oxyphenbutazone/kg of body weight.

zamide dose was examined. In the second study, the effect of varying the oxyphenbutazone doses on tolazamide was analyzed.

The data were analyzed by a two-compartment open model. In the statistical analysis, only three major parameters, α, β, and V_d extrapolated ($V_d$ext), were utilized because they are key factors in explaining a two-compartment open model. Other parameters change with changes in α and β.

Dose-Related Pharmacokinetics of Tolazamide—The pharmacokinetics of tolazamide were studied after intravenous administration of three different doses in each of nine dogs to determine if any dose-dependent changes in tolazamide kinetics were apparent (Table I). The plasma concentration data obtained at each dosage was biphasic (Fig. 1). Therefore, the data were analyzed by a two-compartment open model.

The hybrid rate constants, α and β, and the apparent volume of distribution, $V_d$ext, after intravenous administration of three different dosages of tolazamide in nine dogs are reported in Table II. The hybrid rate constant, α, which represents the rapid distribution of the drug in the body, did not change significantly. Similarly, β, which represents the loss of the drug from the body, did not change significantly with the change in the dosage levels of tolazamide.

A possible explanation for the lack of a significant difference in subject, group, and subject/group could be that mongrel dogs were used and these animals do not represent a genetically controlled breed. The only significant difference observed was in the apparent volume of distribution $V_d$ext at the three dose levels of tolazamide (Table III). It is entirely feasible that $V_d$ext might change without changing α or β as dose increases. Wagner and Pernarowski (5) suggested that the $V_d$ext will vary as a function of dose if the drug has the property of changing its diffusivity or permeability. The pharmacokinetics of tolazamide associated with intravenous administration was dose independent as measured by α and β.

Tolazamide Pharmacokinetics in Presence of Three Oxyphenbutazone Dosages—Results from the pharmacokinetic analysis of the

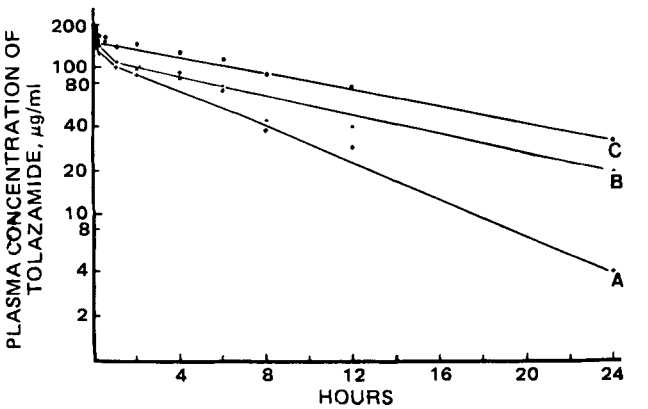

Figure 2—*Plasma concentration* versus *time curves of tolazamide (20 mg/kg of body weight) in presence of oxyphenbutazone for Dog 9. Key: curve A (+), 10 mg of oxyphenbutazone/kg of body weight; curve B (▲), 15 mg of oxyphenbutazone/kg of body weight; curve C (●), 20 mg of oxyphenbutazone/kg of body weight.*

Table V—Statistical Analysis of $V_\beta$ext for Tolazamide at Three Dosages of Oxyphenbutazone

Source of Variation	*df*	*SS*	*MS*	*F*
Total	26	0.0199	—	—
Subject	8	0.0095	0.00119	7.93[a]
Group	2	0.0023	0.00115	7.67[a]
Subject/group	6	0.0072	0.00120	8.00[a]
Week	2	0.0008	0.00040	2.67[b]
Treatment	2	0.0075	0.00375	25.00[a]
Residual	14	0.0021	0.00015	—

[a] Significant at the 1% level. [b] Not significant.

blood concentration data obtained from a dog that received 20.0 mg of tolazamide/kg iv followed by 10.0, 15.0, or 20.0 mg of oxyphenbutazone/kg are depicted in Fig. 2, which shows a biphasic trend. Therefore, blood concentration data were analyzed by a two-compartment open model. Tables IV and V contain the estimated pharmacokinetic parameters and statistical analysis data for these animals.

The parameters α, β, and $V_d$ext obtained from nine dogs after intravenous administration of 20.0 mg of tolazamide/kg followed by one of the three dosages of oxyphenbutazone were analyzed by a three-way analysis of variance. For tolazamide, α and $V_d$ext did not change significantly in the presence of the three oxyphenbutazone doses. However, β was significantly different for tolazamide in the presence of these three oxyphenbutazone doses.

Thus, it can be concluded that oxyphenbutazone only affects the β hybrid rate constant of tolazamide. This effect of oxyphenbutazone appears to be more pronounced as its dose increases. From the data reported here, only a trend can be seen, as depicted graphically in Fig. 3. The decrease in β with increasing dose of oxyphenbutazone represents a decrease in the overall elimination rate of tolazamide from the body. The value for β that was calculated from the first experiment in which the dogs received tolazamide alone cannot statistically be included in the analysis of the second experiment, but it was of the same order of magnitude.

These changes can be explained on the basis of three possible mechanisms of interaction of tolazamide and oxyphenbutazone.

The first possible mechanism involves inhibition of the tolazamide metabolism caused by the presence of oxyphenbutazone. A possible explanation could be that oxyphenbutazone may promote the synthesis of a form of cytochrome P-450 that has a lower metabolic capacity for tolazamide. Induction of this hepatic microsomal monooxygenase system is well established in animals (6). Some inducing agents can also decrease the microsomal oxidative capacity for a substrate (7). Any one or a combination of several of these changes could be the reason for the significant differences in β.

The second possible mechanism involves the displacement of tolazamide from protein binding sites. The values of α and $V_d$ext for tolazamide were not significantly different at the three oxyphenbutazone doses. This finding suggests that there might be little or no competitive displacement of tolazamide from protein binding sites in the presence of oxyphenbutazone.

The third possible mechanism could be inhibition of tolazamide excretion. Many acidic drugs and their metabolites are actively secreted

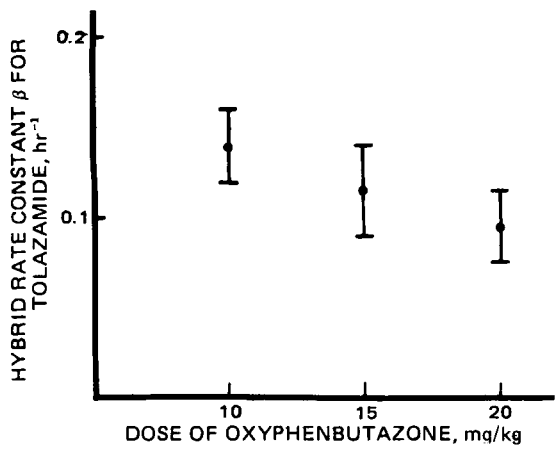

Figure 3—*Mean hybrid rate constant β obtained from nine dogs for tolazamide* versus *dose of oxyphenbutazone.*

by the proximal tubular active transport mechanism, and drug–drug interactions may result from competition with this system. Since tolazamide and oxyphenbutazone are weakly acidic drugs, they might compete for active secretion by the proximal tubular active transport mechanism. The hypoglycemic action of acetohexamide is enhanced by the simultaneous administration of phenylbutazone by the same mechanics (8). Similarly, the hypoglycemic action of tolbutamide is enhanced by the presence of phenylbutazone (9). The value of β for tolazamide seems to decrease with increasing oxyphenbutazone, which could result in an enhanced hypoglycemic effect of tolazamide.

REFERENCES

(1) M. Rowland and S. B. Martin, *J. Pharmacokinet. Biopharm.*, **1**, 553 (1973).

(2) D. A. Hussar, *J. Am. Pharm. Assoc.*, **NS10**, 619 (1970).

(3) L. K. Christensen, J. M. Hansen, and M. Kristensen, *Lancet*, **2**, 1298 (1963).

(4) A. Sedman, and J. G. Wagner, "Manual for Autoan, A Decision-Making Pharmacokinetic Program," Publication Distribution Service, University of Michigan, Ann Arbor, Mich., 1974.

(5) J. G. Wagner, and M. Pernarowski, "Biopharmaceutics and Relevant Pharmacokinetics," Drug Intelligence, Hamilton, Ill., 1971, p. 257.

(6) L. F. Prescott, P. Roscoe, and J. A. H. Forrest, in "Biological Effects of Drugs in Relation to Their Plasma Concentrations," D. S. Davies and B. H. C. Prichard, Eds., University Park Press, London, England, 1973, p. 51.

(7) S. M. Pond, D. J. Birkett, and D. N. Wade, *Clin. Pharmacol. Ther.*, **22**, 573 (1977).

(8) J. G. Field, M. Ohta, C. Boyle, and A. Remer, *N. Engl. J. Med.*, **277**, 889 (1967).

(9) K. F. Ober, *Eur. Clin. Pharmacol.*, **7**, 291 (1974).

ACKNOWLEDGMENTS

Supported by the Research Institute of Pharmaceutical Sciences.

Influence of Food and Fluid Volume on Chlorothiazide Bioavailability: Comparison of Plasma and Urinary Excretion Methods

PETER G. WELLING ˣ and RASHMI H. BARBHAIYA *

Received February 23, 1981, from the *School of Pharmacy, University of Wisconsin, Madison, WI 53706.* Accepted for publication May 13, 1981.

* Present address: Department of Drug Metabolism and Pharmacokinetics, Bristol Laboratories, Syracuse, NY 13201.

Abstract □ The bioavailability of chlorothiazide from oral tablets was examined under fasting and nonfasting conditions in healthy male volunteers. Bioavailability was determined from urinary excretion data and plasma chlorothiazide concentrations. Two fasting treatments and one nonfasting treatment yielded similar plasma chlorothiazide profiles, characterized by sharply ascending and descending segments until 12–13 hr postdosing, followed by a prolonged period with variable and erratic chlorothiazide levels. A triexponential function that adequately described mean data from each treatment could not be applied to individual plasma curves because of their variable nature. Chlorothiazide absorption was not influenced by different accompanying water volumes in fasted individuals but was doubled when tablets were administered immediately after a standard meal. Urinary excretion of chlorothiazide correlated well with plasma drug concentrations; 48-hr urinary recovery accounted for 24.7% of a 500-mg dose in nonfasted subjects compared to 12.3 and 14.9% in fasted subjects receiving the drug with 20 and 250 ml of water, respectively. Observed relationships between chlorothiazide dosage and absorption efficiency are consistent with previous suggestions that chlorothiazide absorption from the GI tract is saturable and site specific.

Keyphrases □ Chlorothiazide—effect of food and fluid volume on bioavailability, plasma and urinary excretion methods compared □ Bioavailability—chlorothiazide, effect of food and fluid volume, plasma and urinary excretion methods compared □ Diuretics—chlorothiazide, influence of food and fluid volume on bioavailability, plasma and urinary excretion methods compared

Chlorothiazide is poorly absorbed after oral doses. Less than 25% of orally administered compound is recovered in urine, compared to >90% following intravenous injection (1–4). Inefficient GI absorption of chlorothiazide may be partly due to its low aqueous solubility but also may be related to saturable and site-specific absorption (5, 6).

The presence of food and the variation in fluid volumes with which a drug is administered can markedly influence drug bioavailability (7–9) with possible clinical consequences (10). The poor and possibly site-specific absorption characteristics of chlorothiazide make it conducive to a bioavailability study under varying dosage conditions.

This report describes chlorothiazide bioavailability following oral doses of commercial tablets to healthy male volunteers in fasting and nonfasting states and with small and large accompanying water volumes. Plasma concentrations and urinary excretion of chlorothiazide were compared using recently described high-pressure liquid chromatographic (HPLC) procedures (11).

EXPERIMENTAL

Subjects—Nine healthy male volunteers[1], 22–33 years of age (mean 27) and weighing 62–88 kg (mean 74), participated in the study after giving informed consent. No subject had histories of drug allergy.

Protocols—Subjects were instructed to take no drugs for 1 week before the study and no drugs other than chlorothiazide during the study. No caffeine-containing beverages were permitted for 1 day before or during the plasma and urine sampling periods following each chlorothiazide dose.

Chlorothiazide[2] was administered as three oral treatments: Treatment A, two 250-mg tablets with 250 ml of water following an overnight fast; Treatment B, two 250-mg tablets with 20 ml of water following an overnight fast; and Treatment C, two 250-mg tablets with 250 ml of water given immediately after a standard breakfast (cornflakes with milk, 150 ml (5 oz) orange juice, two poached eggs, two slices of toast, and one cup of caffeine-free coffee).

Subjects were randomly divided into three groups of three, and the treatments were administered according to a 3 × 3 Latin square design at 1-week intervals. Each treatment was given after an overnight fast; no food, apart from the standard breakfast for those receiving Treatment

[1] Technical staff and graduate students.

[2] Chlorothiazide tablets USP (250 mg), lot 559-197, Lederle Laboratories, American Cyanamid Co., Pearl River, NY 10965.

0022-3549/82/0100-0032$01.00/0