Chlorothiazide Absorption from Solution and Tablet Dosage Forms in Dogs

WILLIAM F. EBLING *, ANDREW F. MURRO *, FRANK J. VOELKER *, DENNIS E. RESETARITS *, and THEODORE R. BATES ‡x

Received March 18, 1980, from the **Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Amherst, NY 14260,* and the ‡*Department of Pharmaceutics, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15261.* Accepted for publication July 30, 1980.

Abstract □ The bioavailability of an aqueous solution of chlorothiazide and three commercially available chlorothiazide tablets was assessed in adult mongrel dogs. In two crossover urinary excretion studies, six fasting dogs received single 500-mg doses of chlorothiazide as an aqueous solution, one 500-mg originator tablet on two separate occasions (Tablets A-1 and A-2), two 250-mg originator tablets (Tablet B), or one 500-mg generic tablet (Tablet C). 6-Amino-4-chlorobenzene-1,3-disulfonamide (chloraminophenamide), a pharmacologically active hydrolysis product of chlorothiazide, was not detected in any urine sample. Urinary recoveries of chlorothiazide after oral administration, expressed as the mean (range) percent of the dose, were only 22.0 (8.41–33.9), 15.7 (10.2–25.0), 20.7 (7.25–31.0), 18.5 (8.72–33.2), and 21.9% (6.69–41.1%) for the aqueous solution and Tablets A-1, A-2, B, and C, respectively. Considerable interindividual variation and some intraindividual variation were observed. No statistically significant difference in bioavailability existed among the aqueous solution and Tablets A-2 and B, between Tablets A-1 and C, and between Tablets A-1 and A-2.

Keyphrases □ Chlorothiazide—bioavailability from tablets and aqueous solution, dogs □ Bioavailability—chlorothiazide, absorption from aqueous solution and tablets, dogs □ Diuretics—chlorothiazide, bioavailability from aqueous solution and tablets, dogs

Chlorothiazide is a diuretic used in the treatment of hypertension, congestive heart failure, and other edematous conditions in animals and humans (1). After intravenous administration to dogs (2–4) and humans (5–7), chlorothiazide is excreted rapidly and completely in the urine as unchanged drug. In contrast, urinary recoveries ranging from only 13 to 32% (percent of dose) have been observed in humans after oral administration of 250–1000 mg of chlorothiazide as tablets (6–9) or as an aqueous solution of the sodium salt (6). Absorption of chlorothiazide in dogs also is poor and variable and apparently is dose dependent at oral doses comparable to those used in the treatment of edematous conditions (4). Thus, the dog appears to be a good animal model for studies designed to assess the influence of physicochemical and physiological factors on chlorothiazide absorption.

The purpose of the present study was to determine the effect of the dosage form and the dosage form formulation on chlorothiazide absorption.

Table I—Experimental Designs for Chlorothiazide Absorption Studies in Mongrel Dogs [a]

Dog	Body Weight, kg	Study I Week 1	Study I Week 2	Study II Week 1	Study II Week 2	Study II Week 3
1	17.0	C	A-1	AS	B	A-2
4	18.5	C	A-1	AS	B	A-2
5	19.7	C	A-1	B	A-2	AS
8	18.1	A-1	C	B	A-2	AS
9	16.6	A-1	C	A-2	AS	B
10	21.8	A-1	C	A-2	AS	B

[a] Each item within the matrix corresponds to a specific formulation. Tablets A-1 and A-2 represent 500-mg tablets (Merck Sharp and Dohme) administered on two separate occasions, Tablet B represents two 250-mg tablets (Merck Sharp and Dohme), Tablet C is one 500-mg tablet (Econo-Rx), and AS is the aqueous solution containing 500 mg of chlorothiazide as the sodium salt.

EXPERIMENTAL

Materials—Chlorothiazide[1], sulfadiazine[2], acetonitrile[3], and ethyl acetate[4] were used as received.

Commercial 250- and 500-mg chlorothiazide tablets[5] manufactured by the originator of the drug product and a generic 500-mg chlorothiazide tablet[6] were obtained commercially. An aqueous solution dosage form of chlorothiazide sodium was prepared immediately prior to use. The 250-mg originator tablet, the 500-mg originator tablet, the 500-mg generic tablet, and the aqueous solution were assayed for their drug content by HPLC and were found to contain 99.5 ± 2.3, 99.4 ± 1.8, 94.1 ± 3.1, and 99.6 ± 1.0% [mean (n = 6) ± SD] of the labeled amount of chlorothiazide. All other chemicals and solvents were reagent grade.

Absorption Studies—Six unanesthetized mongrel dogs (five male and one female), 16.6–21.8 kg (mean 18.6 kg), were fasted with water *ad libitum* for 16–18 hr prior to and for 24 hr after drug administration. Between 8:00 and 9:00 am, each animal received, in two crossover studies (Table I), single 500-mg oral doses of chlorothiazide as one 500-mg originator tablet on two separate occasions (Tablets A-1 and A-2), two 250-mg originator tablets (Tablet B), one 500-mg generic tablet (Tablet C), and 50 ml of an aqueous solution of chlorothiazide sodium. A 1-week washout period was allowed between drug administrations.

Quantitative urine collections were made at 0–12, 12–24, 24–48, 48–72, and 72–96 hr after chlorothiazide administration, and the pH and volume of each collection were recorded. On a separate occasion, 24-hr blank urine specimens were collected from each dog. An aliquot of all specimens was stored at −20° until it was assayed.

The specific HPLC method of Lin and Benet (7) for the quantitative determination of chlorothiazide in urine was used. The original method was modified as described previously (4, 10) to include an extraction step and sulfadiazine as the internal standard.

Differences among more than two mean urinary excretion values were evaluated statistically by an analysis of variance for crossover (Latin square) design, and differences between any two means were compared using either Tukey's multiple-range test or the Student t test for paired samples (11).

RESULTS AND DISCUSSION

There is general agreement that the extent of absorption of thiazide diuretics is assessed best from cumulative urinary excretion data (12–17).

No statistical difference in the urine pH or urine volume existed among the five study conditions. As demonstrated previously (4), chlorothiazide was excreted quantitatively and uniformly in the urine after administration of 50- and 250-mg iv doses of chlorothiazide to four of the six dogs used in the present investigation. Thus, chlorothiazide elimination *via* active renal tubular and biliary secretion (2, 3) apparently occurs in a linear fashion over this dosage range.

The mean cumulative percent of the chlorothiazide dose (based on

[1] Supplied by Merck Sharp and Dohme, West Point, Pa.
[2] American Pharmaceutical Co., New York, N.Y.
[3] High-pressure liquid chromatographic (HPLC) grade, Burdick & Jackson Laboratories, Muskegon, Mich.
[4] Reagent grade, Fisher Scientific Co., Rochester, N.Y.
[5] Diuril tablets, Merck Sharp and Dohme.
[6] Econo-Rx tablets (Bolar). These tablets were from the same lot as those used in the human chlorothiazide bioavailability study performed by Straughn *et al.* (9) and were supplied by these investigators.

0022-3549/81/0200-0224$01.00/0

Table II—Mean Urinary Excretion of Chlorothiazide in Dogs as a Function of Time after Administration of a 500-mg Oral Dose as Two Different 500-mg Chlorothiazide Tablets

Hours	Mean Cumulative Percentage of Dose Excreted ($n = 6$) ± *SD*	
	Tablet A-1[a]	Tablet C[b]
0	0	0
12	8.06 ± 4.1	9.88 ± 5.2
24	10.2 ± 4.4	11.9 ± 7.3
48	15.3 ± 4.8	18.8 ± 8.9
72	15.7 ± 5.1	21.8 ± 11.4
96	15.7 ± 5.1	21.9 ± 11.7

[a] Tablet A-1 represents one 500-mg tablet (Merck Sharp and Dohme) (Trial 1). [b] Tablet C represents one 500-mg tablet (Econo-Rx).

Table III—Mean Urinary Excretion of Chlorothiazide in Dogs as a Function of Time after Administration of a 500-mg Oral Dose as an Aqueous Solution, One 500-mg Tablet, and Two 250-mg Tablets

Hours	Mean Cumulative Percentage of Dose Excreted ($n = 6$) ± *SD*		
	Aqueous Solution[a]	Tablet A-2[b]	Tablet B[c]
0	0	0	0
12	9.30 ± 4.4	10.3 ± 4.8	4.63 ± 4.3
24	11.7 ± 2.4	12.3 ± 5.8	9.29 ± 5.4
48	16.5 ± 4.6	19.4 ± 7.2	16.5 ± 7.8
72	21.3 ± 7.5	20.1 ± 7.1	18.4 ± 7.7
96	22.0 ± 8.0	20.7 ± 6.9	18.5 ± 7.8

[a] Sodium salt. [b] Tablet A-2 represents one 500-mg tablet (Merck Sharp and Dohme) (Trial 2). [c] Tablet B represents two 250-mg tablets (Merck Sharp and Dohme).

assay results) excreted unchanged in the urine at each sampling time after oral administration of the four test dosage forms is shown in Tables II and III. Under all study conditions, plateau excretion values were reached within 48–72 hr, indicating that the experimental period selected was more than adequate to assess the excretion and, thereby, the absorption characteristics of chlorothiazide. As reflected by the high standard deviation associated with each mean excretion value, the urinary excretion rate of chlorothiazide from each dosage form tested showed considerable interindividual variation. This finding is consistent with that observed after oral administration of various brands of chlorothiazide tablets to humans (6, 8, 9).

Thiazide diuretics undergo hydrolysis *in vivo* to 6-amino-4-chlorobenzene-1,3-disulfonamide (I), which is absorbed and excreted in the urine of humans and possesses diuretic activity (18). Although the extent of hydrolysis in humans is greatest for the more potent thiazide diuretics (*e.g.*, cyclothiazide and methyclothiazide) (18), it was important to determine whether I and chlorothiazide were coexcreted in the urine of dogs. HPLC analyses[7] revealed that I was not present in any urine specimen.

Similar to the situation found in humans (6, 8, 9), the extent of chlorothiazide bioavailability in dogs was quite low and variable, regardless of the dosage form and dosage form formulation used (Tables IV and V). Although no statistically significant difference in bioavailability existed between the originator 500-mg tablet (Tablet A-1) and the generic 500-mg tablet (Tablet C), Tablet C appeared to be absorbed more erratically (Table IV). In contrast, Tablets A-1 and C have been reported to be bioinequivalent in humans (9). This apparent species difference may be due to the fact that the human urinary excretion studies were terminated in 24 hr (9), even though chlorothiazide excretion reportedly continues for at least 48 hr in humans (12). It also is possible that the bioavailability estimates for Tablets A-1 and C in humans (9) are in error due to variable interferences from urinary constituents in the modified Bratton–Marshall colorimetric method employed by these investigators (10, 12, 19).

A comparison of the 96-hr urinary recovery values for each dog after administration of Tablet A on two separate occasions (Tablets A-1 and A-2) reveals that the absorption of chlorothiazide also is subject to some intraindividual variation (Tables IV and V). The lack of a significant difference in the mean cumulative 96-hr urinary recovery among the

[7] Under the chromatographic conditions employed (4, 10), the retention times for I, sulfadiazine, and chlorothiazide were 5.25, 5.61, and 6.97 min, respectively.

Table IV—Extent of Chlorothiazide Bioavailability in Dogs after Administration of a 500-mg Oral Dose as Two Different 500-mg Chlorothiazide Tablets

Dog	Cumulative 96-hr Urinary Excretion of Chlorothiazide, mg	
	Tablet A-1[a]	Tablet C[b]
1	61.1	43.7
4	125.2	169.3
5	107.0	122.2
8	51.1	83.4
9	59.3	33.5
10	68.1	205.7
Mean	78.6	109.6
SD	25.4	58.3
CV, %	32	53
Paired *t* test	NS[c] ($p > 0.05$)	

[a] Tablet A-1 represents one 500-mg tablet (Merck Sharp and Dohme) (Trial 1). [b] Tablet C represents one 500-mg tablet (Econo-Rx). [c] Not significant.

Table V—Extent of Chlorothiazide Bioavailability in Dogs after Administration of a 500-mg Oral Dose as an Aqueous Solution, One 500-mg Tablet, and Two 250-mg Tablets

Dog	Cumulative 96-hr Urinary Excretion of Chlorothiazide, mg		
	Aqueous Solution[a]	Tablet A-2[b]	Tablet B[c]
1	76.7	95.1	43.6
4	122.8	155.2	108.4
5	150.5	103.6	113.8
8	97.2	93.3	72.1
9	40.1	36.2	49.9
10	169.7	136.2	165.8
Mean	109.5	103.3	92.3
SD	40.6	34.7	39.1
CV, %	37	34	42

Statistical significance: difference among three oral treatments (analysis of variance for crossover design)
NS[d], $p > 0.05$ ($F = 2.96$; $df = 2,8$)[e]

[a] Sodium salt. [b] Tablet A-2 represents one 500-mg tablet (Merck Sharp and Dohme) (Trial 2). [c] Tablet B represents two 250-mg tablets (Merck Sharp and Dohme). [d] Not significant. [e] Critical *F* value for two and eight degrees of freedom at the 5% level is 4.46.

aqueous solution and the originator 250- and 500-mg tablets (Tablets B and A-2) indicates that chlorothiazide absorption is affected minimally by the nature of the dosage form administered, provided that the dosage form is well formulated.

The results of this study show that at an oral dose comparable to that used in the treatment of edematous conditions in dogs, the absorption of chlorothiazide is variable and incomplete, regardless of whether the drug is administered as an aqueous solution or tablet dosage form. Since the maximum solubility of chlorothiazide at pH 1.0 and 6.2 is 417 and 655 mg/liter, respectively (4), poor drug absorption may have resulted from slow dissolution[8] of the drug due to the high oral dose (500 mg) to solubility ratio employed or from the existence of an active or other capacity-limited intestinal transport process for chlorothiazide (4).

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[8] Upon contacting the acidic gastric fluids, chlorothiazide probably precipitates from the aqueous solution of the sodium salt. Thus, a dissolution step precedes the absorption of chlorothiazide from the solution dosage form.

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ACKNOWLEDGMENTS

Presented in part at the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Washington meeting, April 1980.

Supported in part by Grant GM 20852 from the National Institutes of Health, Bethesda, Md.

Evidence for Metabolic Inertness of Doxycycline

H. J. C. F. NELIS and A. P. De LEENHEER ˣ

Received January 16, 1980, from the *Laboratoria voor Medische Biochemie en voor Klinische Analyse, R.U.G., De Pintelaan 135, B-9000 Gent, Belgium.* Accepted for publication July 17, 1980.

Abstract □ Several conflicting observations in the literature raised considerable doubt about the metabolic fate of doxycycline, which, like other tetracyclines, has been claimed to be metabolically inert. A double liquid chromatographic approach was used in an attempt to demonstrate the polar metabolites and/or conjugates in excreta of human volunteers who ingested the drug. Both ion-exchange and reversed-phase chromatography failed to reveal significant by-products in urine and feces, except for minor amounts of 4-epidoxycycline. In addition, enzymatic hydrolysis procedures did not present any evidence of the conjugates. Thus, the different excretion behavior of doxycycline, compared to other analogs, cannot be explained in terms of increased metabolism.

Keyphrases □ Doxycycline—tetracycline analog, metabolic fate, analysis, human urine and feces □ Liquid chromatography—analysis, doxycycline in human urine and feces □ Antibacterials—doxycycline, metabolic fate, analysis in human urine and feces

It is generally accepted that tetracycline antibiotics are not metabolized in the human body (1–4). However, considerable doubt still exists concerning the fate of the more lipophilic derivatives (5, 6). A study with ^{3}H-labeled doxycycline failed to reveal by-products in excreta, but a rather poor chromatographic technique had been used (7).

Some investigators noted a dramatic decrease in the half-life of the drug following coadministration of typical enzyme-inducing substances, including barbiturates (8), antiepileptics (9, 10), and ethanol (11). This observation was explained in terms of accelerated doxycycline metabolism and led to speculation concerning the formation of conjugates (10). However, other investigators did not support these views and questioned the reliability of the effect (12). Furthermore, an unidentified, biologically inactive fraction is claimed to occur in feces (13–15) and might be due to intestinally formed metabolites.

The lack of suitable chromatographic techniques has hampered the unambiguous settling of these controversial observations. This paper reports the use of liquid chromatography for detecting possible doxycycline by-products in human urine and feces. In addition, enzymatic hydrolysis was carried out in order not to overlook conjugates. Although a similar approach allowed demonstration of minocycline metabolites in urine (16), strong evidence is presented here for the metabolic inertness of doxycycline.

EXPERIMENTAL

Chromatography—Two liquid chromatographs[1], equipped with a sample valve[2] and a variable-wavelength detector[3] operated at 350 nm, were used. The first column (10 × 0.46 cm) consisted of a strong cation-exchange material[4] and was utilized with a 38:62 (v/v) mixture of ethanol–0.1 *M* citrate buffer (pH 4.6) containing 0.05% edetate disodium as the eluent. A reversed-phase system, previously reported for the quantitative determination of doxycycline in human serum and urine (17), was applied to feces samples as well.

Collection of Excreta—A single 200-mg doxycycline dose was administered orally to human volunteers just after a light breakfast. Urine was collected over the following 24 hr. Three days after administration, feces samples were taken. Blank samples were obtained before drug intake.

Extraction of Urine and Feces—Urine samples were extracted with ethyl acetate as described previously (17).

Feces (0.8 g) were homogenized in 0.2 *M* HCl (12 ml) using a high-speed mixer[5]. After centrifugation, a 1-ml aliquot of the supernate was neutralized with 1 *M* NaOH and buffered with 1 ml of phosphate–sulfite buffer (pH 6) (17). Extraction was performed with 10 ml of ethyl acetate. The organic layer was evaporated to dryness[6], and the residue was redissolved in 1 ml of the chromatographic solvent of either the reversed-phase or the ion-exchange system. Finally, 20–100 μl was injected onto the columns. Alternatively, feces were homogenized in 0.2 *M* acetate buffer (pH 5) and processed in a similar way.

Hydrolysis Procedures—Urine samples and feces homogenates were adjusted to pH 5 using acetic acid and 2 *M* NaOH, respectively. Then, they were incubated at 37° with the arylsulfatase–glucuronidase enzyme

[1] Model 8500, Varian Associates, Palo Alto, Calif.
[2] Model CV-6-UHPa-N60, Valco Instruments Co., Houston, Tex.
[3] Varichrom, Varian Associates, Palo Alto, Calif.
[4] Nucleosil SA, 5 μm, Machery & Nagel, Düren, West Germany.
[5] Virtis S 23, Virtis Research Equipment, Gardiner, N.Y.
[6] Rotary Evapo Mix, Büchler Instruments, Fort Lee, N.J.

0022-3549/81/0200-0226$01.00/0