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RELATIVE BIOAVAILABILITY OF ACETAZOLAMIDE TABLETS

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ABSTRACT

Plasma acetazolamide concentrations were determined enzymatically after administration of three tablet dosage forms and a reference solution to 12 human subjects in a crossover study. Two of the tablet products represented different lots from the same manufacturer. There were no significant differences in area under the plasma level–time curves among the four treatments. However, significant differences were found among tablets in terms of peak plasma concentration and time to reach peak concentration. These apparent differences in rate of absorption were correlated with *in vitro* dissolution data obtained in pH 1·5 dissolution medium.

KEY WORDS Acetazolamide Tablets Human bioavailability *In vitro* dissolution Plasma concentrations

INTRODUCTION

Acetazolamide is a potent carbonic anhydrase inhibitor extensively employed in the treatment of glaucoma. Because of its low water solubility, less than $1\,mg\,ml^{-1}$, the drug may have a potential for bioavailability problems. Schoenwald *et al.*[1] compared the absorption of acetazolamide in man using two sustained-release formulations and a reference suspension. The sustained-release capsules were only 30–60 per cent as bioavailable as the suspension. In another study, Yakatan *et al.*[2] evaluated the performance of five lots of acetazolamide 250 mg tablets from the same manufacturer, using a balanced incomplete block design and 20 subjects. They found differences among lots in terms of maximum acetazolamide plasma concentrations in humans given the drug. The differences were correlated with *in vitro* dissolution determined with a rotating-filter-stationary basket device. The U.S. Food and Drug Administration has recently proposed *in vitro* dissolution and *in vivo* bioavailability

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specifications for dosage froms containing acetazolamide and other carbonic anhydrase inhibitors.[3] The purpose of the present study was to examine inter-lot as well as inter-manufacturer variability in the bioavailability of 250 mg acetazolamide tablets. A solution of the drug was used as a reference standard. *In vitro* dissolution studies were also carried out to determine if differences in tablet bioavailability could be related to the dissolution of the dosage form.

MATERIALS AND METHODS

Analytical procedure

The assay of plasma acetazolamide concentrations was based on the enzymatic procedure of Maren,[4] as modified by Yakatan *et al.*[5] All samples were assayed in duplicate within 3 days of collection.

Dissolution studies

The dissolution of the three tablet formulations was determined at 37° using the Hanson paddle method at 50 rev min^{-1} in 900 ml of 0·1 *N*-HCl.[6]

Clinical protocol

Twelve male volunteers gave written informed consent to participate in the study and underwent appropriate urine analysis and hematological testing (CBC and SMA 18/90) to ensure they were in good health. The subjects ranged in age from 21 to 31 years, in height from 173 to 191 cm, and in weight from 70 to 90 kg.

The sequence of dose administration is given in Table 1 and is based on a crossover matrix designed to minimize the influence of any residual or cumulative effects of the preceding doses.[7] The subject initially designated as Subject 5 withdrew from the study after receiving the second dose because of a possible allergic reaction. He was replaced by a new subject who began the study during the third week, and received the four test preparations in the sequence 1, 4, 2, and 3 at weekly intervals. The data for the original Subject 5 are not included in this report. Instead, data for the new Subject 5 were statistically analyzed as though he had received the doses in the sequence presented in Table 1.

All subjects were administered the acetazolamide formulations along with 200 ml of water in the morning following an overnight fast. No food or liquid other than water was permitted until 4 h after ingestion of the dose. The subjects were instructed to avoid any other medication during the period of the study. There was a seven day 'wash out' period between each dose administration.

Five millilitre blood samples were collected in heparinized containers prior to the dose and at 0·5, 1, 1·5, 2, 3, 4, 6, 8, 10, 12, and 25 h after receiving the dose. The blood samples were immediately centrifuged, and the plasma fraction was removed and frozen until assayed.

Table 1. Experimental design for acetazolamide bioavailability study

Subject No.	Week of study*			
	1	2	3	4
1, 2, 3	1	2	4	3
4, 5, 6	2	3	1	4
7, 8, 9	3	4	2	1
10, 11, 12	4	1	3	2

*Each number within the matrix represents a specific product code number:
1—Lederle Laboratories, 500 mg of acetazolamide as the sodium salt for intravenous administration (Lot 490-376) given orally as 275 mg of acetazolamide in solution.
2—Lederle Laboratories, 250 mg tablet (Lot 485-636).
3—Warner-Lambert, 250 mg tablet (Lot 6A001A).
4—Lederle Laboratories, 250 mg tablet (Lot 485-586).

Product selection

The products tested in this study are summarized in Table 1. All tablets were obtained from the U.S. Food and Drug Administration. The reference oral solution was freshly prepared each study day by transferring the contents of two vials of Product 1 to a 100 ml graduated cylinder. The solution was made palatable by adding 1·0 g of sucrose and 23 ml of glycerin, and the mixture was brought up to 91 ml with distilled water.

Each subject received 25 ml of the final solution containing 274·7 mg of acetazolamide. The dose of 274·7 mg rather than 250 mg was inadvertently prepared because of a labelling ambiguity on the parenteral vial which indicated that it contained 500 mg of sodium acetazolamide, whereas it actually contained 500 mg of free acid. Therefore, all data reported for the solution were normalized to a 250 mg acetazolamide dose.

Data analysis

The elimination rate constant for each dosage administration was determined by least-squares fitting of all terminal log–linear concentration time data. The area under the plasma concentration–time curve from 0 to 25 h was calculated using the trapezoidal method. The AUC(0–∞) was calculated using standard methods.[8]

A three-way analysis of variance was used to evaluate statistical significance ($p < 0{\cdot}05$) at each sampling time following the administration of each drug product to each subject each week. In addition, the time of peak plasma

concentrations, peak plasma concentration and area under the plasma concentration–time curve were subjected to the same statistical analysis. In cases where significant differences occurred, the Newman–Keuls *a posteriori* test was used to evaluate which subjects, treatment sequence or dosage forms were different ($p < 0.05$).

RESULTS AND DISCUSSION

Plasma concentration at each sampling time

Mean acetazolamide plasma concentrations at each sampling time for the twelve subjects are summarized in Table 2 and graphically illustrated in Figure 1. At all but the 3 h sampling time, the difference between the product exhibiting the highest concentration and the lowest concentration was greater than 20 per cent. Generally, the relative standard deviation (R.S.D.) of each product at the individual sampling times was in excess of 30 per cent. The solution had a lower R.S.D. than the tablets during the first 2 h, indicating this formulation had less variable absorption among the subjects.

The results of the Newman–Keuls *a posteriori* test given in Table 3 indicated the solution (Product 1) and one tablet (Product 3) exhibited significantly higher ($p < 0.05$) plasma concentrations than at least one of the other tablets for the first three sampling times. At 8, 10, and 12 h mean plasma concentrations with the solution were significantly lower than at least one of the tablets.

Peak concentration, time of peak and area under the curve

The peak plasma concentration, time of maximum plasma concentration, and area under the plasma concentration–time curve (AUC) determined from

Table 2. Acetazolamide plasma levels at each sampling time*

Product No.†	Time (h)										
	0·5	1	1·5	2	3	4	6	8	10	12	25
1	21·0	17·4	13·9	11·3	9·1	7·2	5·3	3·9	3·1	2·4	0·8
	(21)	(24)	(20)	(20)	(34)	(42)	(29)	(37)	(25)	(38)	(70)
2	3·0	8·1	7·6	9·7	10·2	8·6	6·7	5·3	3·8	2·9	1·1
	(93)	(63)	(67)	(36)	(38)	(19)	(26)	(15)	(25)	(25)	(49)
3	14·7	16·4	14·6	13·6	10·9	9·8	6·1	4·5	3·3	3·0	1·0
	(55)	(32)	(37)	(51)	(31)	(36)	(38)	(42)	(36)	(31)	(68)
4	7·0	10·4	11·3	14·4	11·0	9·8	6·9	5·1	4·1	3·2	1·3
	(133)	(70)	(55)	(44)	(52)	(37)	(32)	(30)	(31)	(32)	(46)
% difference‡	86	53	50	33	17	27	23	26	24	25	38

* Each value ($\mu g\,ml^{-1}$) represents the mean of 12 subjects. The relative standard deviation, S. D. × 100/mean, given in parentheses.
† See Table 1 for Product Code numbers.
‡ Percentage differences calculated as [Highest Mean − Lowest Mean] × 100/Highest Mean.

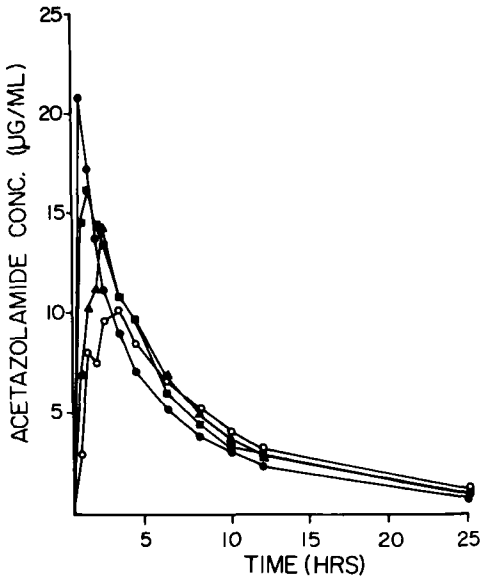

Figure 1. Mean acetazolamide plasma concentrations. Each data point is the mean of 12 subjects. Product 1: ●; Product 2: ○; Product 3: ■; Product 4: ▲

individual subject data are summarized in Table 4. The statistical analysis is presented in Table 3.

The peak plasma concentration ranged from 13·0 µg ml^{-1} (Product 2) to 21·5 µg ml^{-1} (Product 1). This 40 per cent difference was significant ($p < 0{\cdot}05$) with Product 2 having a lower peak concentration than all the other products. There was no difference between Products 4 and 3, but the solution exhibited a significantly higher peak concentration than Products 2 and 4. The variability in the peak concentration was least for the solution as indicated by a R.S.D. of 19 per cent compared to a range of 27 per cent to 36 per cent for the tablets. A study by Yakatan *et al.*,[2] using five lots of 250 mg acetazolamide tablets from the same manufacturer as Products 2 and 4, found peak concentrations ranging from 6·9 µg ml^{-1} to 11·4 µg ml^{-1} as compared to 13·0 µg ml^{-1} and 17·6 µg ml^{-1} for two lots from that manufacturer in the present study. Another study by Yakatan *et al.*[5] showed peak acetazolamide concentrations of 16–17 µg ml^{-1} with a 250 mg dose administered to two subjects, while Wallace *et al.*[9] found peak concentrations ranging from 10 to 18 µg ml^{-1} in a study of 5 subjects. Based on the study of Friedland *et al.*[10] the clinical significance of the observed differences in peak plasma concentrations may be minimal. These workers found that 63 mg oral doses of acetazolamide suspension, which resulted in peak plasma concentrations of 4–5 µg ml^{-1}, were as effective in lowering intraocular pressure as larger doses yielding peak plasma concentrations of 10 µg ml^{-1} or greater. However, these conclusions were based on single dose

Table 3. 'Newman–Keuls *a posteriori* test' for significant differences among products

Parameter	Product Ranking (Lowest to Highest)*†			
(Plasma concentration at)				
0·5 h	2	4	3	1
1 h	2	4	3	1
1·5 h	2	4	1	3
2 h	2	1	3	4
3 h	1	2	3	4
4 h	1	2	3	4
6 h	1	3	2	4
8 h	1	3	4	2
10 h	1	3	2	4
12 h	1	2	3	4
25 h	1	3	2	4
Peak concentration	2	4	3	1
Time of peak concentration	1	3	4	2
AUC (0–25 h)	2	1	3	4
AUC ($0-\infty$) h	1	2	3	4

* Products underlined by a common line not found to differ significantly ($p > 0{\cdot}05$).

† See Table 1 for Product Code numbers.

responses and the author cautions chronic dosing may result in a different dose–response relationship.

The time of peak concentration, which ranged from 0·6 to 2·4 h, was least for the solution as would be expected for this dosage form relative to tablets. The time of peak for the solution was not significantly shorter than one of the tablets (Product 3), although both of these doses exhibited a time of peak significantly less than the other two tablets (Products 2 and 4).

No significant differences were found among the mean area under the plasma concentration–time curves calculated over the 25 h duration of the study or extrapolated to time infinity. It was somewhat surprising to observe the AUC for the solution was lower than or equal to the AUC values seen with the three tablet dosage forms. However, these differences were not statistically significant. Since the initial plasma sample was not obtained until 0·5 h after administration of the solution, it is possible that peak plasma concentrations occurred earlier than 0·5 h, resulting in an underestimation of the AUC for the solution. A previous study[11] also reported a lower AUC for a reference solution compared to two test tablets.

Table 4. Acetazolamide bioavailability parameters* and dissolution characteristics

Product No.†	Peak plasma conc. ($\mu g\,ml^{-1}$)	Time of peak plasma conc. (h)	AUC (0–25 h) ($\mu g\,ml^{-1}\,h$)	AUC ($0-\infty$) ($\mu g\,ml^{-1}\,h$)	Percentage dissolved‡ 20 min	40 min	60 min
1	21·5 (19)	0·6 (48)	102·4 (25)	113·0 (31)	—	—	—
2	13·0 (29)	2·4 (42)	100·7 (17)	113·9 (23)	10·3	15·7	20·7
3	20·2 (27)	1·0 (23)	114·5 (30)	127·4 (31)	60·6	80·9	91·1
4	17·6 (36)	2·0 (57)	115·5 (28)	129·5 (31)	14·7	23·1	29·6

* Each value represents the mean of 12 subjects. Relative standard deviation, S. D. × 100/mean, given in parentheses.
† See Table 1 for Product Code numbers.
‡ Dissolution data previously reported in Reference 3.

This study demonstrated differences in peak plasma concentration between two tablet lots obtained from the same manufacturer, and differences in time of peak concentration among tablets from different manufacturers. It should be noted the formulation of Products 2 and 4 has subsequently been modified by the manufacturer, so the observations of this study are not indicative of the bioavailability of currently marketed lots. These studies do however indicate a need for a bioequivalence requirement for acetazolamide dosage forms since formulation can affect the absorption rate and possibly the therapeutic response of this drug.

Power analysis

Because of the considerable variability in the data, a subject sample size in excess of thirty would have been required to establish a significant difference ($p=0{\cdot}05$) of 20 per cent between products at a number of the sampling times ($\alpha = 0{\cdot}05$, $\beta = 0{\cdot}2$) (Reference 12). For the AUC($0-\infty$) and peak plasma concentrations a difference of at least 25 per cent among products would have been required for significance ($p<0{\cdot}05$). The observed difference in peak plasma concentration was approximately 40 per cent which was statistically significant ($p<0{\cdot}05$).

Half-life

The mean subject half lives for the four dosage forms ranged from $3{\cdot}8 \pm 1{\cdot}6$ h to $9{\cdot}8 \pm 6{\cdot}8$ h. These results are in general agreement with the mean half-life of $8{\cdot}5 \pm 2{\cdot}5$ h reported by Schoenwald *et al.*[1] for 18 healthy human subjects. There were no significant half-life differences ($p>0{\cdot}05$) for treatments or weeks.

In vivo–In vitro *correlations*

The dissolution characteristics of the three tablets are given in Table 4. Product 3 was appreciably more rapidily dissolved than the other two products at each sampling time. A perfect rank-order correlation was observed between percentage dissolution at each sampling time and peak acetazolamide plasma concentration. There was also a perfect inverse rank-order correlation between percentage dissolved and time to achieve peak plasma concentration. The lack of correlation between dissolution and AUC suggests that dissolution of the tablets was related to the rate but not extent of drug absorption *in vivo*. However, Schoenwald *et al.*[1] have reported a correlation between dissolution at pH 1·5 and AUC for sustained release acetazolamide dosage forms. Yakatan *et al.*[2] also have observed a rank-order correlation between dissolution and peak plasma concentration for five tablet lots of acetazolamide. They found a rotating-filter-stationary basket apparatus provided for a better correlation than the U.S.P. rotating basket method. It is recognized that an *in vitro–in vivo* rank-order correlation based on only three dosage forms does not provide sufficient data for a rigorous statistical evaluation of the relationship. However, these data, along with the work of Yakatan *et al.* is adequate to suggest that dissolution testing of acetazolamide dosage forms can provide information which is useful in assessing potential differences in rates of absorption of the drug from different dosage forms. Based largely on this and previous work,[2] a dissolution specification has been proposed by the U.S. Food and Drug Administration.[3] Thus, acetazolamide tablets will be required to exhibit an *in vitro* dissolution rate of not less than 50 per cent in 30 min and not less than 80 per cent in 60 min, using the U.S.P. XX Apparatus 1 at 100 rev min^{-1}, with 0·1 *N*-hydrochloric acid as the dissolution medium.

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