DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 4(6), 599-609 (1978)

DECREASED BIOAVAILABILITY OF SUSTAINED RELEASE ACETAZOLAMIDE DOSAGE FORMS

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ABSTRACT

Bioequivalence comparisons of two sustained release and an immediate release acetazolamide dosage form performed in normal human volunteers (n = 18) demonstrated a large statistical difference between the preparations. The sustained release dosage forms were 40-70% less available than the rapid release form based on comparisons of AUC data. Plasma level data from subjects given a suspension of acetazolamide yielded a biological half-life of 8.5 (± 2.54) hours which is twice that reported previously. A comparison of the AUC data and dissolution profiles generated for each dosage form showed a rank order correlation when a pH 1.2 dissolution fluid was used; however, correlation was not evident when the dissolution media was exchanged for pH 4.5 or 7.3 dissolution media.

INTRODUCTION

The ideal sustained release dosage form provides a rapidly released therapeutic dose, followed by a gradual release of medication over a prolonged period of time. The advantages of a sustained release product over an immediate release dosage form are generally claimed to be improved therapy as a result of "constant" blood levels and/or patient convenience and compliance. In the case of acetazolamide sustained release capsules, the primary advantage appears to be a significant reduction in side effects compared to the conventional tablet. For example, a comparative study of 250 mg acetazolamide tablets given four times a day to 93 patients and 500 mg sustained release capsules given once a day to 83 patients showed that 47% were required to discontinue the tablet medication compared to only 9% for the capsule regimen.[1] Although blood levels were not monitored, the reduction in side effects is likely a result of lowered peak levels associated with the sustained release preparation.[2,3]

The objective of the present study was to compare the bioavailability of a 500 mg test sustained release capsule to a 500 mg sustained release standard and to 500 mg of rapid release acetazolamide given in divided doses of 250 mg. Additionally, it was hoped that correlations could be obtained between bioavailability data and *in vitro* dissolution rate data.

MATERIALS

Three dosage forms were tested for dissolution properties and in human volunteers. They were: Diamox® Sequel® 500 mg (Lederle, Lot 497-662); sustained release test capsules containing 500 mg of drug formulated as slow release granules (Alcon, XE-1345); and acetazolamide, U.S.P. 250 mg (American Cyanamide, Lot 5253), loosely filled into No. 0 gelatin capsules.

METHODS

Dissolution Procedure - The dissolution of each dosage form was performed in a three-necked, 2 liter round bottom flask using 1000 ml of fluid maintained at 37° by immersing the flask in a constant temperature water bath. The fluids were stirred at 175 rpm (Cole Parmer Standard Servodyne Motor). The stirring shaft (E. H. Sargent, size A, S-76636) was positioned through the center part of the three necked flask using the appropriate sleeve for the 24/40 flask connection. Glass stoppers were placed in the other two parts to prevent evaporation. The stirring shaft was equipped with a moon-shaped paddle (E. H. Sargent, size B, 3" length, S-76637), the bottom of which was positioned 2.5 cm from the bottom of the flask. Both simulated gastric (pH 1.2) and intestinal (pH 7.3) fluids without enzymes (N. F. XIV) as well as a mixture of the two fluids (pH 4.5) were used as dissolution fluids. The quantity of drug released into the dissolution fluids was monitored for a total of seven hours. The capsule was first added to gastric fluid and 1.0 ml samples were filter pipetted and assayed at half or one hour intervals through 2 hours. At this time the stirring was stopped, the flask was removed and the gastric fluid including the remaining granules was filtered under pressure using a Buchner funnel. The granules were collected on filter paper and carefully set aside until the apparatus could be reassembled. The pH 3.5 mixture of gastric and intestinal fluids was added to the stirred fluid. Two milliliter samples were filter pipetted, diluted to 50 cc using gastric fluid and assayed every hour for another 2 hour period. The fluid was again filtered after the two hour period to retain the remaining granules which were then added to stirred simulated intestinal fluid at 37°. A one milliliter aliquot was then filter pipetted every hour for 3 hours.

The 1.0 samples were diluted to 50 ml using simulated gastric fluid and assayed spectrophotometrically at 265 nm (Beckman Model 25 Spectrophotometer). A solution of acetazolamide, U.S.P. containing 3-6 µg per ml of simulated gastric fluid was used as the reference standard. Four to six determinations were made for each dosage form; two 250 mg acetazolamide capsules were determined simultaneously.

Bioavailability Study - Eighteen healthy adult male volunteers received each formulation in a randomized, three-way crossover design. The volunteers were between the ages of 20 and 41, without history of chronic obstructive lung or hepatic disease. Laboratory tests of blood chemistry and urine demonstrated a normal range of values. All subjects were within the recommended limits of height-weight proportions.[4] Subjects did not have food for 12 hours prior to receiving the medications. Four ounces of water were taken with each sustained release capsule. A 250 mg acetazolamide dose was administered at time zero and repeated in four hours by emptying the contents of each capsule in 4 ounces of water and drinking the resulting suspension. All subjects were served a standard lunch six hours into the study. A one week washout period was followed for each trial. There were three trials each consisting of three groups, 6 subjects per group receiving a different medication. Subjects were allowed to ambulate freely during the 36 hours following dosing. Initial blood samples (10 ml) were drawn into heparinized tubes immediately before dosing and again at 1, 2, 4, 6, 8, 11, 21, 25, 30 and 36 hours after dosing. The blood samples were centrifuged followed by collection of the plasma. The plasma was frozen immediately and assayed for acetazolomide by an enzymatic method.[5]

RESULTS AND DISCUSSION

Figure 1 shows a comparison of the mean plasma levels for each dosage form. Table 1 summarizes the means standard devia-

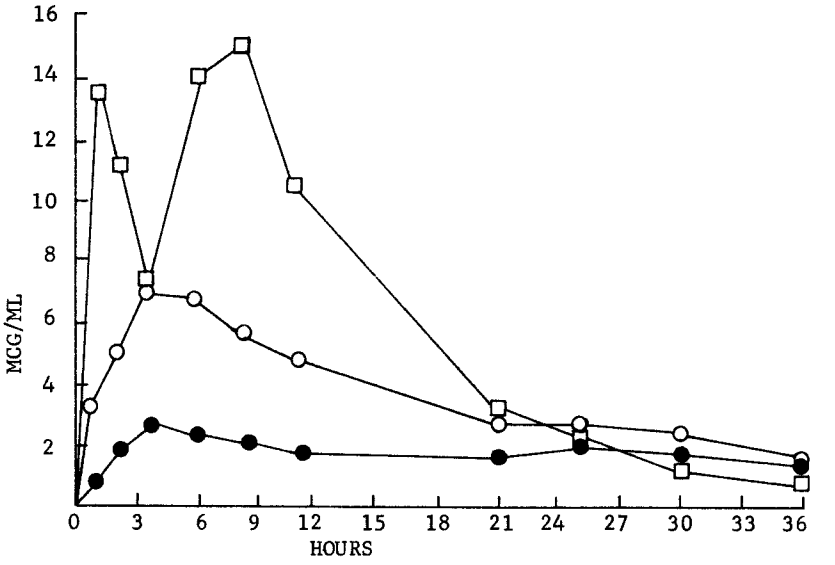

FIGURE 1

A plasma level comparison of Diamox® Sequels® (500 mg, -O-), a sustained release test formulation (500 mg, -●-) and a suspension dosage form (250 mg x 2 doses, -□-).

tions, coefficient of variation and analysis of variance for each dosage form for the area under the plasma-time curves (AUC), the peak concentration, time to peak and all plasma levels. These data demonstrate that statistical differences exist for the three formulations for most of the parameters tested. The AUC results for the 500 mg dosage forms, Diamox® Sequels® and the sustained release test capsule, show a reduced bioavailability when compared to the suspension (250 mg x 2 doses). On a relative basis the AUC results were: 1.0: 0.60: 0.31, for suspension, Diamox® Sequels® and sustained release test capsules, respectively. For Diamox® Sequels®, a comparison of the plasma levels with other published data[2 3 6] shows that the levels associated with

TABLE 1

Summary of Means, Standard Deviations, Coefficients of Variation and Analysis of Variance Results for the Bioavailability Parameters.

	Sustained Release Test Capsule 500m	Diamox® Sequel® 500 mg	Acetazolamide 250 mg x 2(q 4 h)	F-Ratio	p
AUC	68.50 ± 33.05(49)*	133.36 ± 44.76(33)	221.44 ± 51.28(23)	100.46	<0.001
Peak Conc.	3.39 ± 2.00(59)	8.48 ± 2.59(31)	16.89 ± 4.21(25)	107.81	<0.001
Peak Time	9.18 ± 9.92(108)	3.82 ± 3.11(81)	------	2.66	0.092
Plasma Levels:					
1 hr	0.84 ± 0.85(101)	3.35 ± 2.90(87)	13.57 ± 4.17(31)	59.98	<0.001
2 hr	1.91 ± 1.11(58)	5.02 ± 2.13(42)	11.18 ± 3.38(30)	59.82	<0.001
4 hr	2.51 ± 0.73(29)	7.04 ± 2.29(33)	7.30 ± 1.90(26)	55.53	<0.001
6 hr	2.28 ± 0.57(25)	6.80 ± 3.18(47)	14.23 ± 3.74(26)	86.56	<0.001
8 hr	2.03 ± 0.66(33)	5.79 ± 2.90(50)	15.09 ± 3.61(24)	164.01	<0.001
11 hr	1.91 ± 0.63(33)	4.82 ± 2.53(52)	10.51 ± 3.31(31)	78.97	<0.001
21 hr	1.88 ± 1.96(103)	2.71 ± 1.05(39)	3.13 ± 0.98(31)	5.03	0.016
25 hr	2.14 ± 2.19(102)	2.64 ± 0.94(36)	2.18 ± 0.77(35)	0.75	0.482
30 hr	1.86 ± 1.01(54)	2.25 ± 0.91(40)	1.35 ± 0.44(33)	6.81	0.005
36 hr	1.57 ± 0.65(41)	1.87 ± 0.84(45)	0.89 ± 0.35(39)	12.00	<0.001
Coeff. of Var. (Ave ± S.D.)	58 ± 32	47 ± 15	31 ± 4.5		

* Value in parenthesis represents coefficient of variation.

the sustained release capsule are approximately half the values observed for the immediate release dosage form.

It is important to recognize that the apparent decrease in bioavailability of the sustained release acetazolamide dosage forms may have significant therapeutic consequences. There is no question that the sustained release dosage form, Diamox® Sequels®, is an effective therapeutic agent backed by years of clinical acceptance and usefulness. Combining this fact with the decreased bioavailability of the dosage form in relation to a rapid release form suggests that effective dosing of acetazolamide can be accomplished with lower doses than are currently employed. The present bioavailability study suggests that the use of a rapid release dosage form of acetazolamide could be accompanied by a 40% reduction in dose. This thinking is consistent with recent evidence that indicates that low doses of acetazolamide (approximately one-fourth the normal dose) may be as effective in lowering intraocular pressure as the usual doses employed.[7,8] Although these studies were done in small numbers of patients, the data, taken with the present bioavailability data, are very compelling. Significant lowering of acetazolamide side affects should also occur if a lower dose is employed.

Reduced bioavailability of a dosage form means that effectively a lower dose of drug is being administered when that product is utilized. In addition, decreased bioavailability is often accompanied by an increase in variation in blood levels. Both of these factors could lead to variable therapeutic results. In the present study, comparisons of the coefficients of variation for each bioavailability parameter show that as the bioavailability of the dosage form improves, an accompanying reduction in variation can be noted.

Surprisingly, the biological half-life calculated from the suspension plasma levels (21 through 36 hours) was twice the value previously reported, 8.5 (± 2.54) hours vs. 4.1 (range: 2.4-5.8) hours,[9] respectively. The latter value was determined from the plasma levels of 9 glaucoma patients who had received an oral dose of 500 mg of acetazolamide. Six sampling times were divided between twelve hours with one additional plasma sample measured at 24 hours. Not enough determinations were made beyond 12 hours to detect a long distribution phase, therefore, a shorter calculated half-life would be expected.

It is possible that a long distribution phase could be explained by the strong affinity of acetazolamide for carbonic anhydrase located in blood cells and other tissues[10] from which a slow distribution of free drug back into plasma could occur. A similar conclusion was made by Bayne et.al.[2] from a study comparing plasma levels after administration of a 500 mg oral tablet and a 500 mg sustained release capsule. Plasma levels were measured through 45-50 hours. Although a half-life was not reported, it is apparent from the data shown graphically, that the half-life is much longer than four hours for data obtained later than 15 hours after a dose. Plasma levels representing both preparations declined in a parallel fashion beyond 10 hours indicating absorption was completed and that the slowly declining levels were not a consequence of slow release. Maren[10] has shown that the decline of acetazolamide from the plasma of dogs does not reach a steady state until 2 days after dosing.

Figure 2 compares the dissolution results for the three formulations. Sink conditions prevailed throughout the experiment. Consequently, ninety-seven percent of the theoretical amount of drug dissolved from the loosely filled capsules within the first 30 minutes in gastric fluid. The slow release pattern of both sustained release capsules is evident from their profiles. Over

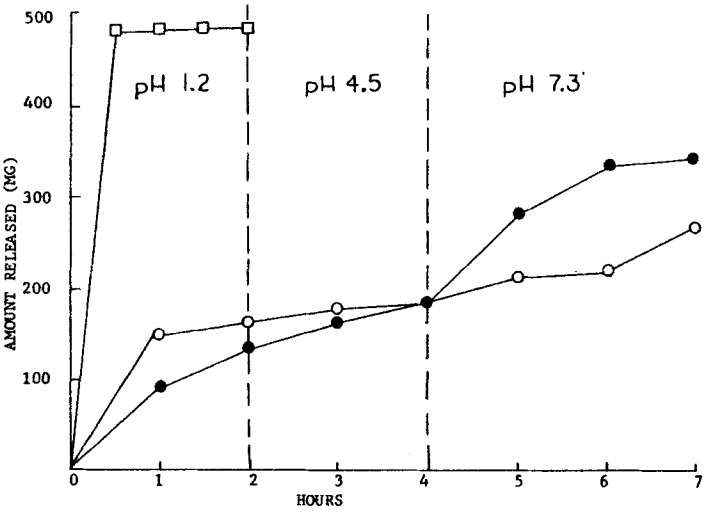

FIGURE 2

An averaged comparison of the amount of acetazolamide released from Diamox® Sequels® (500 mg, -O-, n=4), a sustained release test formulation (500 mg, -●-, n=6) and from a loosely filled No."0" capsule (2 x 250 mg, -□-, n=6). In all cases, one standard deviation was less than 10% of the mean.

the first four hours, and particularly over the first hour, the Diamox® Sequel® releases more drug. However, beyond one hour the release rate for the sustained release test capsule was more rapid. Over seven hours the latter dosage form released 68% of drug, whereas, Diamox® Sequel® released only 54%.

A perfect rank order correlation exists when the amount dissolved for each dosage form through two hours (pH 1.2 fluid) is compared to the AUC of the bioavailability results. Beyond three hours of dissolution (pH 4.5 and 7.3 fluids) a rank order correlation no longer is evident.

Two possibilities exist to help explain the lack of correlation over most of the dissolution profile. Obviously, it must be considered that the dissolution methodology is inadequate in order to correctly describe the unknown *in vivo* release rates. Alternately, a preferential absorption site might exist for acetazolamide. If acetazolamide is absorbed from the upper gastrointestinal tract, then releasing drug after the slow release granules have passed that site would give a decreased extent of absorption when compared to an immediate release dosage form that releases drug before the site is reached.

SUMMARY AND CONCLUSIONS

A comparison of the area under plasma-time curves and dissolution profiles generated for each dosage form showed a rank order correlation when a pH 1.2 dissolution fluid was used; however, correlation was not evident when the dissolution media was exchanged for pH 4.5 or 7.3 dissolution fluid. These latter results illustrate the importance of establishing good *in vivo/in vitro* correlations before *in vivo* extrapolations can be made using dissolution results.

ACKNOWLEDGMENTS

Ms. M. Kellogg is thanked for performing the dissolution work.

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