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Factors Influencing Comparative Bioavailability of Spironolactone Tablets

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Abstract □ The bioavailability of spironolactone from 10 tablet formulations, selected to provide a wide range of specifications and *in vitro* dissolution rates, was assessed from the plasma and urinary levels of its major unconjugated metabolite, canrenone, in a study of balanced incomplete block design using 11 healthy subjects. Significant but weak correlations existed between the amount of spironolactone in solution at 40 min *in vitro* and the area under the plasma concentration–time curve for canrenone and urinary canrenone excretion. The correlations between *in vitro* dissolution and bioavailability parameters appeared to be weakened by two tablet formulations, one with dibasic calcium phosphate as the principal excipient and the other formulated from micronized spironolactone bulk drug. Measurement of *in vitro* dissolution of spironolactone tablets is of value for quality control purposes, provided no major alteration is made in the formulation.

Keyphrases □ Spironolactone—bioavailability in humans related to *in vitro* dissolution, various tablet formulations compared □ Bioavailability—spironolactone in humans, related to *in vitro* dissolution, various tablet formulations compared □ Dissolution, *in vitro*—spironolactone, related to human bioavailability, various tablet formulations compared □ Diuretics—spironolactone, bioavailability in humans related to *in vitro* dissolution, various tablet formulations compared

Spironolactone[1], a synthetic steroid lactone having properties compatible with specific competitive antagonism of aldosterone and other mineralocorticoids (1–3), is of clinical value in the treatment of congestive heart failure, hepatic ascites, primary aldosteronism, and essential hypertension (4). The original tablet formulation of spironolactone had incomplete bioavailability, and reformulation yielded a tablet that proved to be fourfold superior with regard to plasma levels of the principal unconjugated metabolite (canrenone) (5–8), pharmacological activity (9, 10), and therapeutic efficacy (8).

It was suggested that the spironolactone absorption was limited by its dissolution rate, and some evidence indicated that the improved bioavailability of the new formulation could be related to more rapid *in vitro* dissolution (11). The new formulation of spironolactone (25-mg tablets) is still used, and little has been published on spironolactone bioavailability in recent years.

A new 100-mg spironolactone tablet was shown to be bioequivalent to four 25-mg tablets (12). Spironolactone was included in the long list of drugs for which more information on bioavailability is considered desirable (13, 14).

This paper presents the comparative bioavailability of 10 tablet formulations of spironolactone. The objective of the study was to determine whether dissolution *in vitro* is of value in predicting *in vivo* bioavailability and which factors in tablet specification may be important to bioavailability.

[1] Aldactone, G. D. Searle & Co.

EXPERIMENTAL

Formulations—Three experimental and seven production batch tablets were studied. All tablets were chemically equivalent inasmuch as they all complied with the BP monograph requirements for spironolactone. The tablets were selected specifically to provide the wide range

Table I—Properties of the 10 Spironolactone Tablet Formulations Tested

Property	Tablet A	B	C	D	E	F	G	H	I	J
Weight of spironolactone per tablet, mg	25	25	25	100	100	100	100	25	50	100
Weight of tablet, mg	266	266	266	292	664	664	664	664	185	430
Source of spironolactone bulk drug	5	1	3	1	2	4	4	3	7	7
Site of manufacture of tablet	4	4	3	1	3	4	4	5	6	6
Principal excipient[a]	I	I	I	II	I	I	I	I	III	III
Chemical treatment[b]	U	U	U	U	U	U	U	U	M	U
In vitro dissolution, %, at:										
10 min	26	44	40	4	36	19	25	71	21	33
20 min	42	86	48	12	64	42	50	87	57	48
40 min	51	95	61	23	76	57	65	93	71	59

[a] Excipients were calcium sulfate dihydrate (I), dibasic calcium phosphate (II), and lactose (III). [b] M = micronized, and U = nonmicronized.

of specifications and *in vitro* dissolution properties shown in Table I. They were classified by weight of spironolactone per tablet (three categories), weight of tablet (five categories), sites of manufacture of chemical (six sites) and of tablets (five sites), principal excipient (three categories), and particle size (micronized or nonmicronized).

The bioavailability of Tablets B and D was tested in separate studies. Tablet D, a 100-mg tablet, had surprisingly high bioavailability considering its very slow *in vitro* dissolution (Table I) (15) and was included in the present study for this reason. The sparing solubility of the drug, which led to the original bioavailability problem (11), also precluded development of an intravenous formulation. In addition to the 10 tablet formulations, an oral spironolactone solution was included for between-study quality control purposes. Since the spironolactone dose in the solution was lower than that of the tablets, the results for the solution are not relevant to the main study and are not presented.

Dissolution Rate—The dissolution rates of the 10 tablet formulations were determined by a rotating-paddle method. Five liters of USP gastric juice without enzymes was equilibrated to 37° in a three-necked, round-bottom flask. The polytef paddle (132 × 24 mm) was immersed to 2 cm from the bottom of the flask and rotated at 100 rpm.

The amount of drug dissolved from tablets placed at the bottom of the flask was determined from samples removed at 10, 20, and 40 min. After removal, the solution was filtered and spironolactone was determined spectrophotometrically using a standard solution of spironolactone USP as the reference. The percentage dissolution of spironolactone from the 10 tablet formulations at the three sampling times is shown in Table I.

Subjects and Study Design—Eleven male subjects, 21–56 years old, were judged healthy after a medical history, physical examination, and biochemical and hematological screen; they gave informed consent to the study. The design was a balanced incomplete block termed a Youden square (16), with the 11 subjects each taking six of the 11 treatments (10 tablet treatments and one solution) on a single occasion. The six study phases were each separated by an interval of 1 week.

Procedure—The treatments were given with 250 ml of water at 9:00 am following an overnight fast, and the subjects continued fasting for 4 hr after treatment. The dose of the spironolactone tablets was 100 mg as one 100-mg tablet, two 50-mg tablets, or four 25-mg tablets. Venous blood was taken before treatment (for measurement of blank values), at nine timed sampling points in the 6 hr after treatment, and then at 12, 24, 36, 48, 72, and 96 hr. Urine was collected for 96 hr.

Blood samples were placed in heparin lithium anticoagulant and centrifuged within 1 hr, and the separated plasma was stored at −20° until assay. Aliquots of urine were also stored at −20°. The samples were stable over the analysis period as judged by quality control samples of plasma stored under similar conditions at known levels and assayed with each batch of clinical samples. The subjects abstained from alcohol during sampling periods, and all other medication was prohibited throughout the study. The subjects were ambulant and followed their normal diet apart from the periods of fasting.

Analytical—Spironolactone itself does not appear in plasma or urine in measurable quantities; bioavailability was assessed from the levels of its principal unconjugated metabolite, canrenone, in plasma and urine. Canrenone was measured by the fluorometric method of Gochman and Gantt (17). The mean recovery of canrenone from plasma was 75% (range of 62–81%); from urine, it was 98% (range of 93–100%).

From three quality control samples included with the plasma assays, the mean coefficient of variation between batches was 12%. For the urine assay, the between-batch coefficient of variation was 5% (mean for two

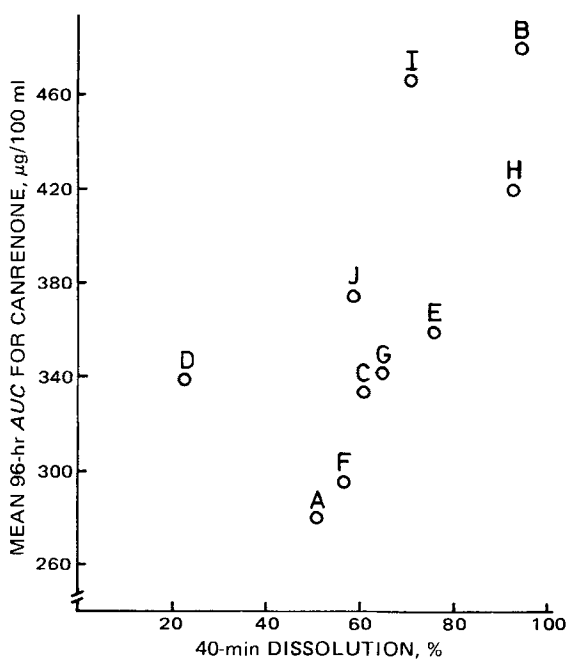

Figure 1—*Plot of AUC for canrenone* versus *40-min dissolution* in vitro.

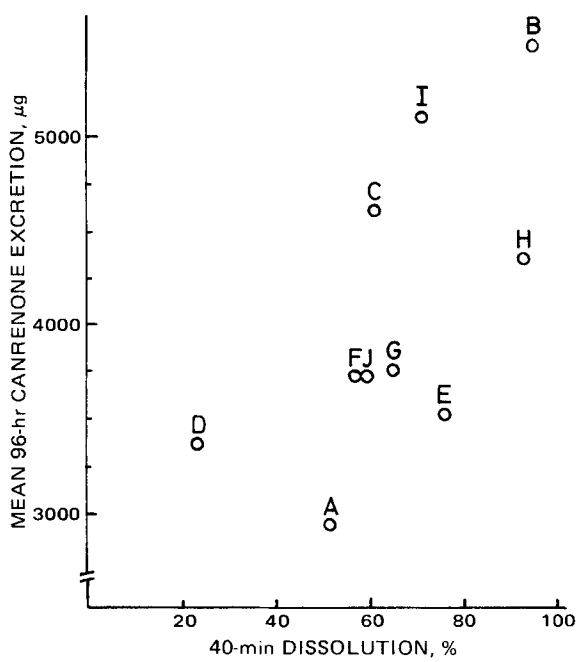

Figure 2—*Plot of mean 96-hr urinary canrenone excretion* versus *40-min dissolution* in vitro.

Table II—Adjusted Mean Results for the Bioavailability Parameters after Administration of 100 mg of Spironolactone in 10 Different Tablet Formulations

Parameter	Tablet A	B	C	D	E	F	G	H	I	J	p^a
Peak plasma canrenone, μg/100 ml	13.1 (2.2)[b]	24.9 (6.7)	16.7 (3.5)	20.0 (4.2)	23.7 (17.4)	17.5 (3.4)	20.2 (5.6)	27.1 (12.6)	29.0 (7.7)	16.6 (6.6)	<0.01
Time to peak canrenone level, hr	3.3 (0.8)	4.0 (1.4)	3.4 (1.2)	2.7 (1.0)	2.6 (0.8)	2.6 (0.8)	3.8 (1.1)	3.0 (1.0)	3.1 (1.2)	3.8 (1.3)	NS
96-hr *AUC* for canrenone, μg/100 ml × hr	267 (113)	478 (214)	332 (241)	338 (55)	349 (193)	296 (106)	341 (219)	420 (102)	465 (378)	373 (272)	<0.05
96-hr urinary canrenone excretion, μg	2960 (1031)	5484 (1279)	4608 (1417)	3385 (963)	3536 (1263)	3721 (1169)	3769 (1468)	4366 (1605)	5105 (1860)	3747 (1078)	<0.01
40-min dissolution, %	51	95	61	23	76	57	65	93	71	59	—

[a] The p values for significance of the differences between the 10 tablet formulations. [b] Figures in parentheses are unadjusted standard deviations with $n = 6$.

quality control samples). The mean ± *SD* of all plasma blank values was 0.63 ± 0.44 μg/100 ml. The plasma blank values did not differ significantly between the six study phases or between the 11 treatments, indicating that the 1-week interval between treatments was adequate and that the blank values had not biased the comparison of the treatments.

Statistical—The parameters of bioavailability analyzed were the peak plasma canrenone level, the time to peak, the area under the plasma concentration–time curve for canrenone from 0 to 96 hr (*AUC* for canrenone), and the total urinary excretion of canrenone over 96 hr. Since plasma canrenone levels often reached the lower limit of assay sensitivity before a terminal log-linear phase of the canrenone concentration–time curve was reached, the *AUC* values were not extrapolated to infinity. The *AUC* for canrenone, calculated by the trapezoidal method, therefore represents the average plasma canrenone level to the finite time of 96 hr.

The differences between the 10 tablet formulations were investigated by the analysis of variance appropriate to the Youden square design (16). Relationships between *in vivo* and *in vitro* measurements were examined by product-moment correlations, and the significance levels given are for two-tailed tests. The mean results of the parameters of bioavailability for each formulation were adjusted for differences between subjects (16).

RESULTS

The adjusted mean (and standard deviation) results of the parameters of bioavailability for the 10 tablet formulations are shown in Table II. The 10 formulations differed significantly regarding peak plasma levels of canrenone ($p < 0.01$), the *AUC* for canrenone ($p < 0.05$), and urinary canrenone excretion ($p < 0.01$) but not regarding the time to reach peak canrenone levels.

Bioavailability Parameters Related to *In Vitro* Dissolution—There were no significant correlations between the amount of spironolactone in solution at 10 min and the bioavailability parameters. The results for spironolactone dissolution at 20 and 40 min were very similar, and only the 40-min dissolution data will be presented (Table III).

The amount of spironolactone in solution at 40 min correlated significantly with the *AUC* for canrenone ($r = +0.66$, $p < 0.05$, Fig. 1) and with urinary canrenone excretion ($r = +0.65$, $p < 0.05$, Fig. 2). The correlation of 40-min dissolution with peak canrenone levels approached significance ($r = +0.61$, $p < 0.1$, Fig. 3), but that with the time to peak plasma canrenone ($r = +0.33$) was not significant ($p > 0.1$).

In Fig. 3 and, to a lesser extent, in Fig. 1, it can be seen that the correlations are improved if two of the 10 formulations, Tablets D and I, are excluded. There was a suggestion that these two formulations gave peak canrenone levels and *AUC* values for canrenone that are relatively high for a given dissolution. Examination of Table I shows that Tablets D and I were unique with regard to tablet weight, and Tablet I was also different with respect to the amount of spironolactone per tablet (50 mg). These variables did not appear to influence the relationship between *in vitro* dissolution and bioavailability for the other tablet formulations and are not considered to be the most probable causes of the findings. Tablet D was the only formulation with dibasic calcium phosphate as its principal excipient, and Tablet I was the only one formulated from micronized spironolactone bulk drug; these factors may be responsible for the different behavior of these formulations.

Table III—Correlations of the Amount of Spironolactone in Solution at 40 min with the Bioavailability Parameters for All 10 Spironolactone Tablets ($n = 10$) and Excluding the Results for Tablets D and I ($n = 8$)

40-min Dissolution *versus*	$n = 10$ r	p	$n = 8$ r	p
Peak plasma canrenone	+0.61	<0.1	+0.95	<0.001
Time to peak canrenone	+0.33	NS[a]	+0.13	NS
96-hr *AUC* for canrenone	+0.66	<0.05	+0.90	<0.005
96-hr urinary canrenone excretion	+0.65	<0.05	+0.72	<0.05

[a] NS = $p > 0.1$.

Recalculation of the correlation coefficients excluding the results for Tablets D and I (Table III) showed high correlations of the 40-min dissolution with the *AUC* for canrenone ($r = +0.90$) and peak canrenone level ($r = +0.95$) and a moderate correlation with urinary canrenone excretion ($r = +0.72$). There was still no correlation with the time to reach peak plasma canrenone concentration.

Variability between Subjects in Bioavailability Parameters—In Table IV the range of adjusted mean results observed between the 10 tablet formulations is compared to the range of adjusted mean results between the 11 subjects. The variability between subjects at least equaled that between formulations for each parameter. For the *AUC* for canrenone, the intersubject differences were considerably greater than the differences between formulations.

DISCUSSION

The marked and significant differences between the 10 tablets with regard to the *AUC* for canrenone in plasma and the urinary excretion of canrenone indicate that the total amount of spironolactone absorbed from different formulations may vary greatly. The adjusted mean 96-hr *AUC* for canrenone for the different formulations ranged from 267 to 478 μg/dl hr. Such a wide range was not observed among spironolactone tablets actually released for marketing; the formulations tested in this study were

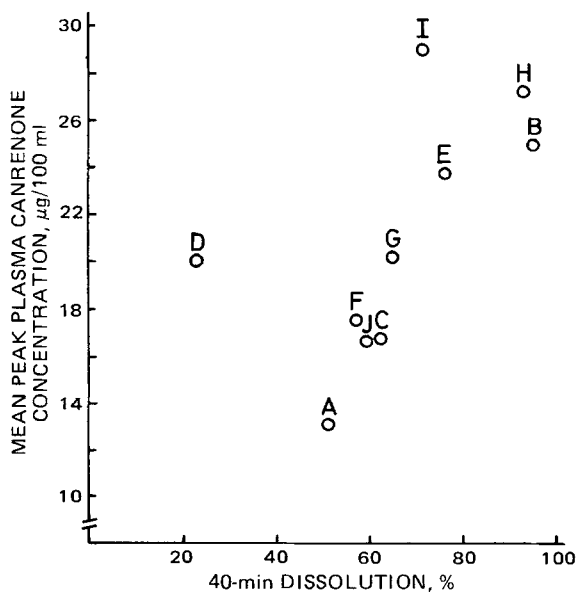

Figure 3—*Plot of mean peak plasma canrenone concentration* versus *40-min dissolution* in vitro.

Table IV—Range of Adjusted Mean Results Observed Comparing the Variability between 10 Spironolactone Formulations with the Variability between 11 Subjects

Parameter	Range of Observations	
	Between Formulations	Between Subjects
Peak plasma canrenone, μg/dl	13.1–29.0	14.7–31.7
Time to peak canrenone, hr	2.6–4.0	2.4–4.4
96-hr *AUC* for canrenone, μg/dl hr	267–478	231–878
96-hr urinary canrenone excretion, μg	2960–5484	2422–5119

selected specifically to provide variability and included three experimental preparations. In addition, the *in vitro* dissolution parameters of spironolactone tablets were already in use for quality control purposes prior to this study on a purely empirical basis. The present study shows that spironolactone bioavailability is potentially very variable, and as discussed below, gives some guidance as to how this variability can be controlled.

The present results confirm the suggestion of Levy (11) that the amount of spironolactone absorbed from tablet formulations is related to their dissolution characteristics. No such correlation was found in the earlier study of Tidd *et al.* (15), but this result is not surprising in view of the design of that study and the number and range of the formulations examined. In the present study, there were significant correlations of the amount of spironolactone in solution at 40 min *in vitro* with the *AUC* for canrenone and with urinary canrenone excretion and a similar trend with the peak plasma canrenone concentration. The range of times to reach peak canrenone levels was small, and the differences between the formulations were not significant. These results may account for the fact that the *in vitro* dissolution did not correlate with the time to reach peak canrenone levels, the parameter expected to relate most closely to the absorption rate of spironolactone.

The correlation between *in vitro* dissolution and the *AUC* for canrenone is important in confirming that spironolactone bioavailability is dissolution related (11), but it leaves a lot to be desired from the practical point of view. Ideally, such a correlation should be robust, so that the relationship holds despite even major alterations in tablet specification, and should have a coefficient high enough to allow reasonably accurate prediction of *in vivo* properties from the *in vitro* dissolution profile (18). With the results for all 10 formulations, the correlations observed fell short of these criteria. From the correlation coefficient for 40-min dissolution *versus* the *AUC* for canrenone ($r = +0.66$), it can be calculated that only 44% ($r^2 \times 100$) of the variability in the *AUC* for canrenone between formulations could be attributed to differences in 40-min dissolution. Although the observed correlation is probably an underestimate of the true correlation due to the experimental errors in the observed values of 40-min dissolution and particularly the mean *AUC*, part of the variability in the *AUC* values between formulations probably must be attributed to factors other than *in vitro* dissolution, as measured in the present study.

Inspection of the data gives some information suggesting the nature of these other factors and also suggests that the correlations between *in vitro* dissolution and *in vivo* parameters may be of value for quality control purposes. Certain factors in tablet specification had little or no influence on bioavailability independent of any effect on *in vitro* dissolution, including the weight of the tablet, the site of manufacture of spironolactone bulk drug, and the site of manufacture of the tablet. Similarly, the weight of spironolactone per tablet did not appear to influence bioavailability, supporting a previous report that four 25-mg tablets and one 100-mg tablet of spironolactone were bioequivalent (12).

However, two tablet formulations may have weakened what was otherwise a high correlation between *in vitro* dissolution and the *AUC* for canrenone. These findings may be attributed to a difference in the principal excipient for one tablet and to micronization of the spironolactone bulk drug in the other, although other factors in these formulations could equally be responsible. In any event, the results for these formulations indicate that the correlation between *in vitro* dissolution and bioavailability is not impervious to all changes in tablet specification. When the results for these formulations were excluded, the correlations of 40-min dissolution with the *AUC* for canrenone and the peak plasma canrenone concentration were considerably improved. Therefore, the *in vitro* dissolution of spironolactone tablets is likely to have value as a quality control procedure, particularly when there are no major changes in tablet specification. When the specifications are altered, for example by changing the particle size or a major excipient, reevaluation of the bioavailability by an *in vivo* study would be desirable.

The two tablets (D and I) that appeared to deviate from the relationship between *in vitro* dissolution and *in vivo* bioavailability are of interest. Formulation D was remarkable in having high bioavailability despite its extremely low *in vitro* dissolution (23% spironolactone in solution at 40 min). For some reason, the dissolution of this tablet in the GI tract may be more rapid than its *in vitro* dissolution. The high bioavailability of Tablet I may be attributed to micronization of the spironolactone bulk drug. Reduction in particle size has been shown to improve the bioavailability of other compounds with low water solubility, such as griseofulvin (19) and digoxin (20), and a similar effect from micronization previously was suggested for spironolactone (7). However, reduction in particle size would be expected to increase bioavailability through an increase in the dissolution rate of the drug (18), and it is not clear why the bioavailability of the micronized spironolactone tablet was higher than would be predicted from the *in vitro* dissolution rate.

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