Oral Absorption with Various Preparations of Spironolactone in Dogs

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Gastrointestinal absorption of various spironolactone preparations was investigated in acute tests with dogs treated with an aldosterone-like steroid. Absorption was determined by the appearance in plasma of aldadiene, a major spironolactone metabolite with fluorogenic properties, and by renal manifestations of mineralocorticoid antagonism. Variable absorption was noted with tablet forms of spironolactone with particle size controlled to suggest a profound influence of pharmaceutical formulation. Available data would indicate that the assay using dogs is valuable for clinical prediction of absorption differences between spironolactone preparations.

SPIRONOLACTONE[1] is a steroidal spirolactone (1) with the ability to inhibit competitively the renal electrolyte effects of aldosterone (2, 3). Therefore, it has been of interest as a diuretic agent in clinical edema characterized by hyperaldosteronism (4–6). Recently, clinical evidence was presented to suggest differences in gastrointestinal absorption of various pharmaceutical formulations of spironolactone, as judged by the concentration of its fluorogenic Δ^6-metabolite, aldadiene, in plasma (7–9) or by renal properties of the preparations (7, 8, 10–14). Noteworthy in these observations is that relatively poor absorption occurred with commercial tablets of spironolactone, which contrasted with the view developed independently from data on mineralocorticoid-blocking properties of the pure drug in the laboratory (2). All these observations have suggested the need for further studies on absorption characteristics with various pharmaceutical formulations of spironolactone. This report indicates that satisfactory absorption data may be derived from plasma aldadiene and urinary electrolyte measurements in acute tests with dogs.

EXPERIMENTAL

Healthy female mongrel dogs (14 to 19 Kg. body weight), maintained under constant laboratory conditions of temperature (22–25°) and humidity (25–50%), were used for the experiments. A standard diet consisting of 300 Gm. of homogenized dog meal reconstituted to contain 75 meq. Na was fed each day to the animals; water was provided *ad libitum*. All animals had been previously used for other tests and were acquainted with procedures of bleeding from the jugular vein and bladder catheterization. Spironolactone given either as the milled powder or tablet form was administered orally to dogs treated with a mineralocorticoid, deoxycorticosterone acetate (DCA). Each animal was injected intramuscularly in the thigh with 0.25 mg. of DCA in oil at the time of oral treatment with spironolactone, then placed in a metabolism cage for 6 hours. At intervals of 2 hours, a urinary specimen was obtained by catheterization, followed by a 6-7-ml. blood sample from the jugular vein using a heparinized syringe. Preliminary time-response tests with spironolactone (powder or tablets) indicated that there was no advantage in extending the 6-hour period of assay, considering the time factor for maximal plasma aldadiene response and schedule of a working day. DCA was used in conjunction with spironolactone treatment to induce a relative hyperaldosterone-like state, a condition required for demonstrating renal properties of a mineralocorticoid antagonist. DCA alone was expected to reduce the ratio of urinary Na/K, a frequently used criterion of its aldosterone-like activity (15), by inducing Na retention and K loss. Antimineralocorticoid effects were measured by reversal of the Na/K response to DCA. Spironolactone was given in gelatin capsules as the milled powder, as whole 100-mg. tablets[2] wherein spironolactone is compacted in the tablet core, or as whole 25-mg. uncoated tablets[3] which differ in pharmaceutical compounding from the press-coated tablets by dispersion of the drug in tablets with a water-soluble matrix. Both tablet forms were prepared from the powder of spironolactone, with mean particle diameter of 20 μ measured by a Coulter counter. Standard U.S.P. disintegration tests have revealed that the uncoated and coated tablets of spironolactone disintegrate in 1.5 and 20–30 minutes, respectively. Aldadiene concentration in plasma was determined by a fluorometric technique using the Aminco-Bowman spectrophotometer (16), and urinary Na and K determined on the Beckman flame photometer. Relative potencies of various spironolactone preparations were calculated by the method of Irwin as discussed by Pugsley (17).

RESULTS AND DISCUSSION

Table I describes the mean plasma aldadiene and urinary Na/K response following oral administration of spironolactone (powder form) in animals treated with DCA.[4] In general, increasing doses of spironolactone induced progressively higher levels of plasma aldadiene at each 2-hour interval of measurement; accordingly, the aldadiene level over the total period of test, 0–6 hours, determined by averaging the values obtained at 2, 4, and 6 hours for each of the animals, showed a stepwise increment with dosage of spironolactone. Noteworthy in these 0–6-hour data is that each doubling of the dose produced an average change of 0.28 units (antilog of which is 1.9) or approximately a twofold increment, to indicate a direct proportionality between dosage and plasma level of aldadiene. Furthermore, the time-response data demonstrate that maximal levels of plasma aldadiene were achieved more frequently in the fourth hour of study than with measurements

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[1] 3-(3-Oxo-7α-acetylthio-17β-hydroxy-4-androsten-17α-yl)-propanoic acid lactone.

[2] Marketed as Aldactone by G. D. Searle and Co., Chicago, Ill.

[3] Marketed as Aldactone-A by G. D. Searle and Co., Chicago, Ill.

[4] Transformation of the original values to logarithms provided homogeneous variances with the various treatments as determined by Bartletts' test (18) and, thereby, a statistically useful index for evaluation of the data.

TABLE I.—PLASMA ALDADIENE CONCENTRATION AND URINARY Na/K RESPONSE FOLLOWING ORAL ADMINISTRATION OF GRADED DOSES OF SPIRONOLACTONE IN DOGS TREATED WITH A MINERALOCORTICOID

Treatment Total Dose, mg. DCA	Spironolactone	Animals, No.	Plasma Aldadiene[a] 2 Hr.	4 Hr.	6 Hr.	0-6 Hr.	Urinary Na/K[b]
...	...	9	...	...	...	...	1.36
0.25	...	6	...	...	...	...	0.77[c]
0.25	25	6	0.79	0.86	0.69	0.78	0.93
0.25	50	6	0.64	1.27	1.12	1.01	1.02
0.25	100	9	1.46	1.57	1.42	1.48	1.23[d]
0.25	200	6	1.66	1.60	1.53	1.60	1.26[d]
0.25	400	6	1.61	2.02	2.01	1.88	1.56[d]

[a] Mean log micrograms of aldadiene per 100 ml. plasma; pooled s = 0.232 at 2, 4, or 6 hours (degrees of freedom or DF = 60) and 0.210 at 0–6 hours (DF = 20). [b] Mean log (Na × 10)/K in the 0–6-hour measurement. [c] $p < 0.05$ relative to untreated controls (pooled s = 0.268, DF = 23). [d] $p < 0.05$ relative to DCA alone.

TABLE II.—RELATIVE POTENCIES OF VARIOUS SPIRONOLACTONE PREPARATIONS GIVEN ORALLY IN DOGS TREATED WITH DCA

Assay	Spironolactone	Relative Potency[a] Plasma Aldadiene	Urinary Na/K
A	Powder form	1	1
	Uncoated tablets	0.99 (0.45 to 2.18)	0.81 (0.31 to 2.12)
B	Coated tablets	1	1
	Uncoated tablets	6.68 (2.81 to 15.89)	3.73 (1.50 to 9.20)

[a] Potency (95% confidence interval), as judged by the 0–6-hour measurements; X^2-tests for parallelism of the dose-response curves indicated validity of the assays ($X^2 \leq 0.878$, which is far below value of 3.841 required for significance at p of 0.05).

at 2 or 6 hours. To circumvent the problem of quantitating time-response data statistically, the 0–6-hour response—an index determined from multiple measurements—was selected as the criterion for the assay of absorption differences. The effects of spironolactone on the ratio of urinary Na/K in the total period of test are summarized in the table, together with reference data of untreated and DCA-treated controls. DCA alone gave a relatively low ratio of Na/K; but, co-administration of spironolactone in graded doses progressively reversed the response to DCA, with significant inhibition occurring at the 100, 200, and 400-mg. doses. These data demonstrate the association of antimineralocorticoid effects and plasma aldadiene levels, not unlike that found in man (7, 8) and suggest the value of using dogs for absorption studies with spironolactone.

Quantitative data on absorption differences with the spironolactone powder form and uncoated tablets using procedures of the 0–6-hour assay are summarized in Table II (*Assay A*). In this evaluation both preparations were tested simultaneously at two dosage levels predetermined by preliminary tests and the experiments replicated to obtain crossover data using a group of nine animals. With a weight dosage consideration of spironolactone, the uncoated tablets showed a relative potency that was statistically indistinguishable from that of the powder form of spironolactone, judged by plasma aldadiene levels and urinary Na/K. Interpretation of these data suggests that absorption was either complete for the tablet and powder form or, with particle size and disintegration time presumed equal, rate-limited by a common dissolution property. In contrast, similar data described an important difference in absorption characteristics between the two tablet forms of spironolactone witnessed by data in *Assay B, e.g.*, the uncoated tablets demonstrated a potency of approximately 7 and 4 relative to that of the coated tablets, using plasma aldadiene and urinary Na/K, respectively, as the criterion of absorption. The difference of potencies was highly significant in both comparisons ($p < 0.01$). Thus, it would appear from the available data that pharmaceutical formulation, expressed possibly by ultimate differences in disintegration and/or dissolution rates of the tablets (8, 19), may be an important variable for absorption characteristics of coated tablets and uncoated tablets of spironolactone. The laboratory data with these tablet forms parallel closely those of clinical studies based on plasma aldadiene levels (8, 9), mineralocorticoid-antagonism (10, 11), and diuretic efficacy in edematous patients (12–14).

The assay procedures in dogs provide, therefore, a convenient means for study of absorption differences with various pharmaceutical preparations of spironolactone. Validity of the procedures is in good agreement with the laboratory data and clinical observations.

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