COMPARISON OF PRORENOATE POTASSIUM AND SPIRONOLACTONE AFTER REPEATED DOSES AND STEADY STATE PLASMA LEVELS OF ACTIVE METABOLITES

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1 After repeated single daily doses, the aldosterone antagonists prerenone and spironolactone were compared with regard to renal antimineralocorticoid activity, plasma potassium concentration and steady state plasma levels of their active metabolites, prorenone and canrenone respectively, in a balanced crossover study of twelve healthy subjects.

2 Following challenge with the mineralocorticoid, fludrocortisone, best estimates of the potency of prerenone relative to spironolactone were 3.6 (95% confidence limits 1.6–10.4) for urinary sodium excretion and 3.4 (95% confidence limits 2.0–6.5) for urinary $\log_{10}$ 10 Na/K. Estimates with respect to urinary potassium excretion and plasma potassium concentration were imprecise, confirming the limitations of the fludrocortisone model in the evaluation of aldosterone antagonists at steady state.

3 Both compounds exhibited directly proportional relationships between daily dose and steady state plasma levels of active metabolites. The approximate mean terminal elimination half-life of prorenone at steady state was 32.6 h (range 18–80 h).

Introduction

Spironolactone (Aldactone®) is a competitive aldosterone antagonist which is used clinically as a diuretic with potassium sparing properties (Ochs *et*

al., 1978). Prorenone potassium (Figure 1) is structurally closely related to spironolactone and is a potent mineralocorticoid antagonist in animals

Figure 1 Structures of (a) spironolactone, (b) prorenone potassium, and their active metabolites (c) canrenone and (d) prorenone.

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(Hofmann *et al.*, 1975), and in man (Levine 1976; Ramsay *et al.*, 1975b). In its ability to reverse the renal electrolyte effects of fludrocortisone in healthy humans, prorenone potassium is significantly more potent than spironolactone after single doses (Ramsay *et al.*, 1975a, 1976), the advantage being attributed largely to its more pronounced antikalium-uretic activity (Ramsay *et al.*, 1975a). However, this information may not be representative of the clinical situation where aldosterone antagonists are prescribed for prolonged periods. Comparisons after repeated dosing in the fludrocortisone model, using the dose ratio approach, have suggested that relative potency is significantly greater at steady state than after single doses but have revealed the limitations of that bioassay since valid comparisons with respect to potassium sparing properties could not be made (McInnes *et al.*, 1981; Ramsay *et al.*, 1977a).

In an attempt to determine the potency of prorenone potassium relative to spironolactone after repeated doses, this study reverted to conventional pharmacological principles, the aim being to produce a parallel line bioassay. The drugs were compared with respect to their renal antimineralocorticoid activity and, in view of the information from previous studies (McInnes *et al.*, 1981; Ramsay *et al.*, 1975a) and the clinical role of aldosterone antagonists, potassium sparing properties were studied in detail. Plasma concentrations of prorenone, the only known active metabolite of prorenone potassium (Claire *et al.*, 1979; Funder *et al.*, 1974, 1976) and canrenone, quantitatively the major active metabolite of spironolactone in men (Karim *et al.*, 1976a) (Figure 1), were measured comprehensively to establish that steady state conditions had been reached and to ensure that there were no important qualitative differences in the respective plasma concentration-time curves.

Methods

Rationale

Renal antimineralocorticoid activity was assessed by a modification of the method of Ross (1962). In healthy men, oral administration of the synthetic mineralocorticoid, fludrocortisone, results in sodium retention, increased potassium excretion and depression of the urinary sodium: potassium (Na/K) ratio. The log fludrocortisone-urinary electrolyte response curves exhibit parallelism with those of aldosterone and repeated doses of aldosterone antagonists reverse these changes by increasing natriuresis, decreasing kaliuresis and increasing the urinary Na/K ratio. The fludrocortisone dosage regimen employed was similar to that used previously to study the pharmacodynamics of single doses of spironolactone and prorenone potassium (Ramsay *et al.*, 1976).

Plasma potassium responses were compared at steady state, before and after fludrocortisone.

Subjects

Twelve healthy male subjects aged 18–35 years were studied after medical history, physical examination, laboratory screening and written enquiry of their general practitioners. Written consent for the study was obtained after full explanation of the procedure and its risks. Alcohol was forbidden from 24 h before until 48 h after each treatment period. Drugs other than those tested were prohibited from one week before the study until its completion. The protocol was approved by an independent ethical committee.

Study design

Each subject took each of four treatments for 10 days during four study periods separated by 12 day intervals. Fludrocortisone was administered as three equal doses on day 10. Trial drugs were randomly allocated but treatment sequences conformed to a balanced Latin-square design.

Drugs

Prorenone potassium 10 mg and 40 mg (10 mg tablets) and spironolactone 25 mg and 100 mg (25 mg tablets).

The treatments were dispensed double-blind, with matching placebo tablets as required, once daily for 10 days.

Fludrocortisone (Florinef, Squibb) 0.5 mg (5×0.1 mg tablets) was prescribed on three occasions on day 10.

Procedure

From days 1 to 10 of each treatment period, mineralocorticoid antagonist was taken once daily at 09.00 h with 300 ml water. On day 10, fludrocortisone was given with 150 ml water at 07.00 h, 17.00 h and 23.00 h. All urine was collected from 09.00 h to 09.00 h the next day (day 11). Venous blood for estimation of plasma potassium, canrenone and prorenone concentration was taken without forearm compression and with minimal stasis on days 1, 9, 10 and 11 at 09.00 h, and on days 9 and 10, between 10.00 h and 17.00 h, at hourly intervals. In addition, 48 h after the last treatment, samples for plasma prorenone assay were taken from the six subjects who received prorenone potassium during the final phase of the study. Laboratory screening (full blood count, plasma electrolytes, urea and liver function tests) was carried out on blood samples taken on days 1 and 11 of study periods, and 1 week after each period. From 07.00 h on day 10 until

09.00 h on day 11, diet and fluid intake were constant within subjects but not between subjects. Foods with high sodium content were avoided and added salt was not allowed at the table. The subjects were ambulant but avoided strenuous exercise.

Laboratory measurements

Urine was collected without preservatives. After measurement of volume, an aliquot was taken for assay of sodium concentration by atomic absorption spectrometry, and potassium and creatinine concentration by Technicon Autoanalyser (SMA6/60). Plasma was separated within 1 h and stored at -20°C until analysis. Plasma potassium concentration and screening parameters were measured by conventional laboratory procedures. Plasma canrenone concentration was assayed by a fluorimetric method (Gochman & Gantt, 1962) and plasma prerenone by radioimmunoassay using an antibody produced by rabbit in response to the bovine serum albumin conjugate of the 3-carboxymethylxine derivative of prerenone and utilising albumin-coated charcoal to separate the free from antibody-bound antigen.

Statistical analysis

Responses were compared by the analysis of variance appropriate to the Latin-square design which allows for between subject and between phase variation. Between treatment variation in electrolyte effects was partitioned to test the differences between the two dose levels of each compound, the significance of the average slope and the departure from parallelism. Provided the average slope was significant ($P < 0.05$) and there was no contraindication to parallelism, the potency of prerenone potassium relative to spironolactone was regarded as valid and estimated with 95% confidence limits. Urinary potassium concentration was corrected for creatinine concentration to minimise the effects of inaccuracies in urine collections. The findings of significant positive effects on urine volume but negative effects on potassium concentration and $\log_{10}$ 100 potassium/creatinine necessitated analysis of covariance with adjustment to a constant urine volume. In each case this approach was justified by a significant reduction in the error variance. Logarithmic transformation of the parameters normalised the distribution and stabilised the variance. The areas under the plasma concentration-time curves (AUC) for canrenone and prerenone over 24 h from 09.00 h on days 9 and 10 were calculated by the trapezoidal method. AUC for each metabolite at the two dose levels were compared after logarithmic transformation. The ratios of AUC with 95% confidence limits were calculated from the geometric means. The statistical methods used are described by Armitage (1971).

Results

Urinary electrolytes

The mean responses, between subject standard errors (s.e.) and the results of statistical tests are shown in Table 1. Estimation of relative potency with 95% confidence limits are presented in Table 2.

Significant log dose-response trends were apparent following prerenone potassium for urine volume ($P < 0.05$), sodium excretion ($P < 0.005$) and $\log_{10}$ 10 Na/K ($P < 0.001$), and after spironolactone for $\log_{10}$ 10 Na/K ($P < 0.01$). There were slight decreases in potassium excretion at the higher dose levels of both compounds but the differences did not approach statistical significance. Similar trends were apparent with respect to $\log_{10}$ 100 potassium/creatinine and analysis of covariance with urine volume revealed an overall significant difference ($P < 0.01$). The slope of the average log dose-response relationship was significant for sodium excretion ($P < 0.001$), $\log_{10}$ 100 potassium/creatinine after adjustment for urine volume ($P < 0.01$) and $\log_{10}$ 10 Na/K ($P < 0.001$). In the absence of any contraindication to parallelism between the relationships for each drug, valid estimates of relative potency with 95% confidence limits were possible for sodium excretion (3.6, 95% confidence limits 1.6–10.4), adjusted $\log_{10}$ 100 potassium/creatinine (5.4, 95% confidence limits 2.1–51.0) and for $\log_{10}$ 10 Na/K (3.4, 95% confidence limits 2.0–6.5).

Plasma potassium

The mean results for plasma potassium concentrations on days 9 and 10 were very similar suggesting that steady state conditions had been achieved but dose-response relationships were shallow. The mean (s.e. mean) results on day 11, post fludrocortisone, are given in Table 1. There was a significant dose-dependent increment in plasma potassium concentration after prerenone potassium ($P < 0.025$) and the average slope was significant ($P < 0.025$) with no contraindication to parallelism. Best estimate of the potency of prerenone potassium relative to spironolactone was 4.1 with 95% confidence limits 1.0–194.0 (Table 2).

Plasma levels of drug metabolites

Before treatment, plasma canrenone concentrations varied between 0.5 and 1.6 $\mu\text{g}/100\text{ ml}$, and plasma prerenone concentrations were less than 8 ng/ml, the lower limit of sensitivity of the assay, in ten of the twelve subjects. In neither case was there evidence to suggest carry-over effects. Geometric mean plasma canrenone and prerenone concentrations following repeated doses of spironolactone and prerenone

Table 1 Mean (and s.e. mean) results in twelve subjects for 24 h urine electrolyte excretion and plasma potassium concentration after ten days continuous treatment with spironolactone and prerenate potassium

	Spironolactone			Prerenate potassium			P value for:		Deviation from parallelism
	25 mg	100 mg	P* value	10 mg	40 mg	P* value	Difference between four treatments	Average slope	
Urine volume (ml)	1399 (173)	1574 (177)	> 0.1	1542 (122)	1787 (189)	< 0.05	0.025	< 0.025	> 0.1
Sodium excretion (mmol)	98.2 (12.8)	126.4 (13.6)	< 0.1	98.6 (11.4)	145.0 (15.5)	< 0.005	< 0.005	< 0.001	> 0.1
Potassium excretion (mmol)	103.2 (7.3)	99.6 (7.1)	> 0.1	108.4 (6.5)	96.1 (7.1)	> 0.1	> 0.1	> 0.1	> 0.1
Log ₁₀ 100 potassium/creatinine	1.78 (0.02)	1.77 (0.03)	> 0.1	1.79 (0.02)	1.73 (0.02)	< 0.05	> 0.1	< 0.1	> 0.1
†Log ₁₀ 100 potassium/creatinine	1.79	1.77	> 0.1	1.79	1.71	< 0.005	< 0.01	< 0.01	> 0.1
Log ₁₀ 10 Na/K	0.93 (0.05)	1.08 (0.03)	< 0.01	0.93 (0.05)	1.17 (0.05)	< 0.001	< 0.001	< 0.001	> 0.1
Plasma potassium (mmol/l)	3.88 (0.07)	3.93 (0.06)	> 0.1	3.84 (0.08)	4.07 (0.06)	< 0.025	0.05	< 0.025	> 0.1

* P value for significance of difference between doses.

† Adjusted to urine volume of 1575 ml (overall mean) using analysis of covariance.

Table 2 Best estimates of the potency of prrenoate potassium relative to spironolactone on a weight basis with 95% confidence limits for urine electrolyte responses and plasma potassium

	Relative potency prrenoate potassium : spironolactone	95% confidence limits
Urine sodium excretion	3.6	1.6–10.4
*Urine log ₁₀ 100 potassium/creatinine	5.4	2.1–51.0
Urine log ₁₀ 10 Na/K	3.4	2.0–6.5
Plasma potassium	4.1	1.0–194.0

* Adjusted to urine volume of 1575 ml (overall mean)

potassium respectively are shown in Figure 2. Mean peak levels of plasma canrenone were 8.5 µg/100 ml (25 mg spironolactone) and 25.9 µg/100 ml (100 mg spironolactone), and trough levels were 1.9 µg/100 ml and 5.1 µg/100 ml respectively. Mean plasma prrenoate peaks were 117.6 ng/ml and 468 ng/ml for 10 mg and 40 mg prrenoate potassium respectively, and the corresponding trough levels were 23.6 ng/ml and 95.9 ng/ml. The ratio of AUC for 40 mg/day and 10 mg/day prrenoate potassium was 4.04 (95% confidence limits 3.78–4.32) and, after adjustment for baseline levels, the AUC ratio for 100 mg/day and 25 mg/day spironolactone was 3.81 (95% confidence limits 3.24–4.48). For both drug metabolites, AUC on day 10 were lower than those on day 9 and for plasma prrenoate, the mean difference of 4.6% was significant ($P < 0.001$). The approximate mean terminal $T_{1/2}$ in the six volunteers who took prrenoate potassium in the final phase of the study (Figure 3) was 32.6 h (range 18–80 h).

Side effects

A number of minor side effects were noted, particularly after fludrocortisone. No abnormalities of clinical importance were noted in the laboratory tests.

Discussion

In general, the urinary electrolyte findings confirm our previous experience with the drugs (McInnes *et al.*, 1981). The natriuretic potency of prrenoate potassium relative to spironolactone (3.6, 95% confidence limits, 1.6–10.4) was neither statistically inconsistent with the results from the dose-ratio approach (4.2, 95% confidence limits, 2.7–6.9) (McInnes *et al.*, 1981) nor contraindicated a two-fold increase in relative potency with regard to single dose studies (Ramsay *et al.*, 1975a, b).

As in studies using the dose ratio approach (McInnes *et al.*, 1981; Ramsay *et al.*, 1977a), an acceptable estimate of relative potency for potassium excretion was precluded although a statistically valid estimate was possible for urinary potassium concen-

tration, after correction for creatinine concentration (5.4, 95% confidence limits 2.1–51.0). This depended on allowance being made for a mineralocorticoid antagonist dose related effect on urine flow rate which may have an important influence on potassium excretion (Ramsay *et al.*, 1976, 1977b). The wide confidence limits of this estimate indicate its imprecision, a consequence of the shallow aldosterone antagonist dose-response trends. It seems unlikely that this resulted from excessive mineralocorticoid challenge in view of the results for urinary sodium excretion and the similar potassium responses with lower doses of fludrocortisone in previous studies (McInnes *et al.*, 1981; Ramsay *et al.*, 1977a). At steady state, dose-responses for potassium excretion may indeed be shallow or confounding homeostatic influences may conceal the true relationship. Continuous administration of aldosterone antagonists leads to increased endogenous aldosterone secretion so that activity after repeated dosing in the presence of fludrocortisone is a complex mixture of intrinsic pharmacological activity at renal level and the effect of the induced state of hyperaldosteronism.

In contrast to earlier work (McInnes *et al.*, 1981) for urinary log₁₀ 10 Na/K, generally considered the best single index of renal mineralocorticoid (Johnson, 1954) or antimineralocorticoid activity (Gantt & Dyniewicz, 1963; Kagawa, 1964), this study allowed a precise estimate of relative potency (3.4, 95% confidence limits, 2.0–6.5). However, the urinary potassium findings and the similarity of this estimate with that for sodium excretion lead us to question the acceptance of this variable alone as an overall index of renal antimineralocorticoid activity.

At steady state the plasma potassium results revealed evidence of treatment effects but differences were slight and did not allow valid estimation of relative potency. However, these influences were more marked on day 11 suggesting significant positive log aldosterone antagonist dose-plasma potassium response relationships in conditions of mineralocorticoid excess, and allowing a valid estimate of relative potency, prrenoate potassium: spironolactone, of 4.1 (95% confidence limits 1–194). The imprecision of this estimate reflects the shallow dose-response

Figure 2 (a) Plasma canrenone concentrations after repeated doses of spironolactone 25 mg (○), and 100 mg (●). (b) Plasma prerenone concentrations after repeated doses of prerenone potassium 10 mg (□) and 40 mg (■). Drugs given once daily at 09.00 h; last treatment on day 10. Geometric mean (vertical bars, asymmetric limits), results in twelve subjects.

relationships, which might be anticipated if mineralocorticoids and their antagonists influence plasma potassium as a consequence of their action on potassium handling at renal level although there is evidence that aldosterone antagonists may have potassium sparing effects at non-renal sites (Jick *et al.*, 1963; Schlienger *et al.*, 1977).

The plasma concentrations of canrenone and prerenone after repeated doses of spironolactone and prerenone potassium respectively were consistent with the assumption that pharmacological steady state had been achieved. The lower AUC for both drug metabolites on day 10 may be explained by the administration of fludrocortisone and dietary control

Figure 3 Plasma prorenone concentration-time curves for 48 h after final dose of prorenone potassium, 10 mg (O) and 40 mg (●). Geometric mean results in three subjects at each dose.

imposed between the sampling points. Within the dose range studied, a directly proportional relationship existed between the daily dose of aldosterone antagonist and steady state concentration of drug metabolites (AUC canrenone ratio 3.81, 95% confidence limits, 3.24–4.48; AUC prorenone ratio 4.04, 95% confidence limits, 3.78–4.32). The wider confidence limits for the canrenone results reflect differences in assay precision and marked inter-subject variability in plasma canrenone concentration (Karim *et al.*, 1976c; Ramsay & Hettiarachchi, 1981; Sadee *et al.*, 1974).

This study provides a comprehensive description of the steady state plasma levels of the active metabolite of each aldosterone antagonist, extending previous less complete information (Karim *et al.*, 1976c; McInnes *et al.*, 1981; Ramsay *et al.*, 1977a; Ramsay & Hettiarachchi, 1981; Sadee *et al.*, 1974). Peak plasma levels occurred 2–3 h after the daily dose of each drug confirming evidence from single dose studies (Karim *et al.*, 1976b; unpublished observations) and the ratio of minimum to maximum steady state plasma levels was about 20% in each case, indicating that both drugs displayed rapid gastrointestinal absorption and that appreciable blood levels were present 24 h after treatment. The approximate estimate of the terminal $T_{1/2}$ of prorenone at steady state (mean 32.6 h, range 18–80 h) was in general agreement with estimates after single doses (unpublished observations), providing little indication that prorenone potassium induces its own metabolism, as has been reported for spironolactone (Solymoss *et al.*, 1970). There was no evidence of dose-dependent kinetics. The considerable inter-individual variability in $T_{1/2}$ is common

to many drugs eliminated chiefly by metabolism where differences can often be attributed to variable rates of metabolism. The complexities of the distribution and metabolism of prorenone potassium have yet to be established but are unlikely to be less than those of spironolactone which undergoes extensive biotransformation (Karim *et al.*, 1976a).

The results of this study confirm the limitations of the fludrocortisone model in the evaluation of aldosterone antagonists under steady state conditions (McInnes *et al.*, 1981). While taking into consideration pharmacokinetic characteristics, estimates of relative potency with regard to potassium handling were imprecise and of doubtful clinical significance. A valid, precise, estimate of relative potency was possible for urinary $\log_{10}$ 10 Na/K, usually considered the best index of renal antimineralocorticoid activity, but this reflects natriuretic potency and should not be considered to give meaningful information concerning antikaliuretic activity. However, for all parameters examined, prorenone potassium was significantly more potent than spironolactone on a weight basis. Since *in vitro* studies (Claire *et al.*, 1979; Funder *et al.*, 1976) have indicated that prorenone potassium may have fewer antiandrogenic effects than spironolactone at a given dose, suggesting an advantageous dissociation between desired and unwanted effects, prorenone potassium may deserve further evaluation. The long $T_{1/2}$ of prorenone at steady state and the significant trough levels lend support to once daily prorenone potassium dosing regimens.

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