Improved bioavailability and clinical response in patients with chronic liver disease following the administration of a spironolactone: β-cyclodextrin complex

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Aims To compare the absorption and clinical effect of spironolactone from an inclusion complex with β-cyclodextrin (SP-COMP) to Aldactone tablets (ALD) in chronic liver disease.

Methods Patients, admitted with chronic liver disease, completed a randomized crossover steady state study. They received their spironolactone dose as either daily SP-COMP or ALD for 7 days. Serial blood samples were drawn over a 24 h period from day 7 of each therapy. Accurate fluid balance was recorded on days 5–7 and 12–14. Thirteen (six females) whose mean (s.d.) age and weight was 58.4(9.3) years and 74.3(19.0) kg completed the study.

Results The mean (95% confidence limits) relative bioavailability for SP-COMP (compared with ALD) from steady state serum concentrations of canrenone, 6β-hydroxyl 7α-thiomethyl spironolactone and 7α-thiomethyl spironolactone was 310.0 (265.4, 336.7), 233.4(212.9, 250.8) and 254.8(230.8, 279.0)%, respectively. Improvements in clinical status and fluid balance occurred over the last 3 days of SP-COMP with a mean (s.d.) net loss, in fluid balance, of 1370(860)ml compared with a gain of 228(936)ml during ALD.

Conclusions Better absorption of spironolactone from the spironolactone: β-cyclodextrin complex formulation should lead to a reduction in dosage and perhaps a more consistent effect in patients with chronic liver disease.

Keywords: spironolactone, β-cyclodextrin, liver disease, bioavailability

Introduction

Spironolactone is a steroidal aldosterone antagonist that has been widely used for almost 30 years in disorders associated with primary or secondary hyperaldosteronism. At present it is mainly used as a diuretic to control ascites in chronic liver disease. It has a low and erratic bioavailability due to poor aqueous solubility which restricts its dissolution rate [1]. This leads to fluctuating clinical control in compliant patients thereby complicating their management [2, 3]. Different formulations have been used to improve the spironolactone oral bioavailability using simultaneous administration of a surface active agent, polysorbate 80 [4], and by reduction of the particle size [3, 5]. Bioavailability studies using micronised spironolactone compared with Aldactone tablets (Searle Pharmaceuticals, USA) in healthy volunteers have revealed a higher C_{max} and large AUC (area under the curve) for the micronised product with a 14% increase in the oral relative bioavailability.

Recently, attention has centred on the formation of inclusion complexes of spironolactone with cyclodextrins. α, β and γ-cyclodextrin contain six, seven and eight glucose units, respectively, in a ring formation which has a hydrophilic outer layer and a relatively hydrophobic inner cavity into which a drug can be incorporated. They are formed by the action of the bacterial enzyme cycloglycosyl transferase (e.g. *Bacillus macerans*, *Klebsiella pneumoniae*) on starch [6]. The formation of α, β and γ-cyclodextrin is dependent on the bacterial source of the enzyme [7]. Inclusion complexation of spironolactone with β-cyclodextrin [8–11] and γ-cyclodextrin [7] has been reported.

The first report of an enhanced bioavailability for spironolactone complexes with β and γ-cyclodextrin was following oral administration to dogs [10]. There was a 2–3 fold increase in the serum canrenone concentrations at 90 min post spironolactone complex dose compared with spironolactone. When an inclusion complex of spironolactone with β-cyclodextrin was orally administered to rats there was an increase in their urinary volume compared with either spironolactone or β-cyclodextrin alone [9]. The first bioavailability study of a spironolactone: β-cyclodextrin complex in humans revealed that significantly higher quantities of fluorimetrically measured canrenone were excreted in the urine after oral administration of the complex than after spironolactone [12]. Using a single dose crossover design study in volunteers we have measured serum canrenone concentrations. The relative bioavailability (using the AUC) of a spironolactone: β-cyclodextrin complex was

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252.0(19.6)% compared with Aldactone 100 mg tablets [13]. We have now extended our bioavailability studies with the administration of spironolactone: β-cyclodextrin complex to patients admitted to hospital with chronic liver disease.

Methods

Preparation of the spironolactone: β-cyclodextrin complex (SP-COMP)

The SP-COMP was formulated as a 1 : 4 ratio of spironolactone: β-cyclodextrin. *In vitro* studies showed that there was an eight fold increase in the aqueous solubility of spironolactone for the SP-COMP compared with spironolactone. Capsules containing 125 mg of SP-COMP (equivalent to 25 mg spironolactone) were manufactured. Dissolution studies using simulated gastric fluid (US Pharmacopoeia Monograph for spironolactone tablets) revealed that after 30 min 99.6($\pm$2.84)% of the spironolactone had dissolved compared with 47.8($\pm$2.76)% for Aldactone tablets ($n=10$).

Patients and samples

Ethics committee approval was obtained from St James's University Hospital, Leeds (UK). Patients admitted with chronic liver disease who gave written informed consent were recruited into the study. The patients had chronic liver disease secondary to either primary biliary cirrhosis, chronic alcohol intake or chronic active hepatitis. Their medication on admission included spironolactone. Those whose management plan was to maintain the drug therapy of admission with daily monitoring continued the study, otherwise they were excluded. They were randomized to receive their daily (morning) dose of spironolactone at 06.00 h (before breakfast) as either the spironolactone: β-cyclodextrin capsules (SP-COMP) containing the equivalent of 25 mg spironolactone, or Aldactone 100 mg tablets (ALD). After 7 days of continuous drug therapy blood samples were drawn via an in-dwelling heparinised cannula, at 0, 0.5, 1, 2, 4, 8, 12, 16 and 24 h post dose. The patients were then crossed over to the other preparation and after a further 7 days of constant oral therapy blood samples were drawn as before. All blood samples were allowed to clot, centrifuged and the serum was separated and stored at -20° C until the time of analysis.

The medical team, responsible for the management of each patient, and the analysts, assaying the samples, were not informed which formulation the patient was taking.

Clinical measurements

Daily weight, accurate fluid balance, urinary volume and clinical examination were recorded. Serum and urinary creatinine, urea and electrolyte concentrations together with liver function tests were measured daily.

Serum metabolite concentration measurement

Serum concentrations of spironolactone (SP), canrenone (CAN), 7α-thiomethyl spironolactone (7-TH) and 6β-hydroxy 7α-thiomethyl spironolactone (6-OH) were measured by high performance liquid chromatography (h.p.l.c.) with solid phase extraction.

Bond Elut 100 mg C18 columns (Jones Chromatography, UK) were conditioned using 1ml methanol followed by 1ml water. Serum (1ml) was placed on the column with 0.3 ml megesterol acetate (10 $\mu g\,ml^{-1}$)—the internal standard—and drawn through the column for 3 min using a Vac Elut vacuum system (Jones Chromatography, UK). Columns were washed with 1ml water and dried for 3 min by the application of a vacuum. The spironolactone metabolites were then eluted from the column using 1ml methanol. This final elute was evaporated to dryness under a gentle steam of nitrogen and the residual analytes were reconstituted in 0.3ml of (the h.p.l.c.) mobile phase prior to injection.

The stationary phase of the h.p.l.c. system was a 4.6 mm (internal diameter) × 15cm Spherisorb C18 column with a 2cm Spherisorb C18 pre-column (Jones Chromatography, UK). The mobile phase was 65% methanol in water at a flow rate of 1.0 min^{-1}. A Gilson UV model 116 dual detector (Anachem, UK) was set at 240nm for the detection of spironolactone, 7α-methyl spironolactone and 6β-hydroxy 7α-thiomethyl spironolactone and at 280nm for canrenone and megesterol acetate. Reconstituted sample (100 μl) was injected into the system for each assay.

Analysis of the data

The AUC at steady state was calculated using the linear trapezoid rule between the serum concentrations of the blood samples taken before the study dose ($t=0$) and at $t=$ 24 h (immediately before the next morning's dose). C_{max} was taken to be the highest serum concentration of the blood samples and the time of this occurrence was the t_{max}. Half-lives were not calculated from the terminal slope of the serum concentration data because of the lack of a sufficient number of samples in the terminal phase.

Statistical comparisons of the AUC, C_{max} and t_{max} data were made by the Wilcoxon signed rank test and the median difference with their 95% confidence intervals were calculated. For each metabolite profile the serum concentration at $t=0$ was compared with that at $t=24$ h using a paired *t*-test to demonstrate steady state status.

Results

Following solid phase extraction of SP, CAN, 6-OH, 7-TH and megesterol acetate (1.S) from standard serum samples the % recovery was 92, 94, 93, 92 and 95% respectively. The h.p.l.c. retention times were 11.1, 9.1, 8.2, 13.3 and 17.9 min. All the peaks, on the chromatogram, demonstrated baseline resolution and analysis of blank serum revealed no interfering peaks. The accuracy and precision of the assay are shown in Table 1. All calibration curves were linear.

Thirteen patients (six females) completed the study. Their mean (s.d.) age and weight was 58.4(9.3)years and 74.3(19.0)kg, respectively, and their daily (morning) spironolactone dosage was 242.3(70.7)mg with a range of 200–400 mg. Serum and urinary concentrations of creatinine, urea and electrolytes revealed that no patient was in renal failure. The serum potassium and sodium concentrations were all within normal limits.

Table 1 Accuracy and precision data of the h.p.l.c. assay for spironolactone (SP), canrenone (CAN), 6β-hydroxy 7α-thiomethyl spironolactone (6-OH) and 7α-thiomethyl spironolactone (7-TH) ($n=10$ on all occasions).

Serum concentration	*Intra-day CV (%)*	*Inter-day CV (%)*	*Mean (s.d.) (% of nominal concentration)*
(a) SP 25 μg ml^{-1}	3.4	5.3	101.2(4.4)
800 μg ml^{-1}	2.8	2.4	97.5(3.2)
(b) CAN 60 μg ml^{-1}	10.2	9.5	95.8(4.2)
2600 μg ml^{-1}	2.8	2.6	98.4(3.4)
(c) 6-OH 50 μg ml^{-1}	8.3	6.9	96.6(1.3)
1600 μg ml^{-1}	5.6	2.3	96.4 (4.1)
(d) 7-TH 100 μg ml^{-1}	9.8	8.7	96.2(4.6)
2600 μg ml^{-1}	4.8	6.5	96.3(3.2)

CV—coefficient of variation.

Serum spironolactone concentrations were undetected in nine of the patients during dosing with Aldactone tablets. Figures 1a, b and c show that the serum concentrations of CAN, 6-OH and 7-TH, respectively, were greater during the administration of SP-COMP than ALD. Statistical analysis (paired *t*-test) between the serum metabolite concentration for each profile at $t=0$ and $t=24$ h after the administration of each formulation revealed no significant difference. Table 2 shows that the AUC and C_{max} of CAN, 6-OH and 7-TH were significantly greater during the administration of SP-COMP than ALD. The mean (s.d.) relative bioavailability of the SP-COMP (compared with ALD) using the AUC data for CAN, 6-OH and 7-TH was 301.0 (58.9), 233.4(35.2) and 254.8(39.9)%, respectively. The 95% confidence limits of these ratios was 265.4–336.7, 212.9–250.8 and 230.8–279.0. Analysis of variance demonstrated that there were no period effects nor treatment-period interactions.

Following daily clinical examination, all patients were noted to have improved on day 7 of SP-COMP therapy but not ALD. Figure 2 shows that this is demonstrated by a marked diuresis in most patients during the last 3 days of SP-COMP administration. The mean (s.d.) fluid balance over the last 3 days of SP-COMP and ALD therapy was −1370 (860) and +228 (936)ml respectively ($P<0.001$).

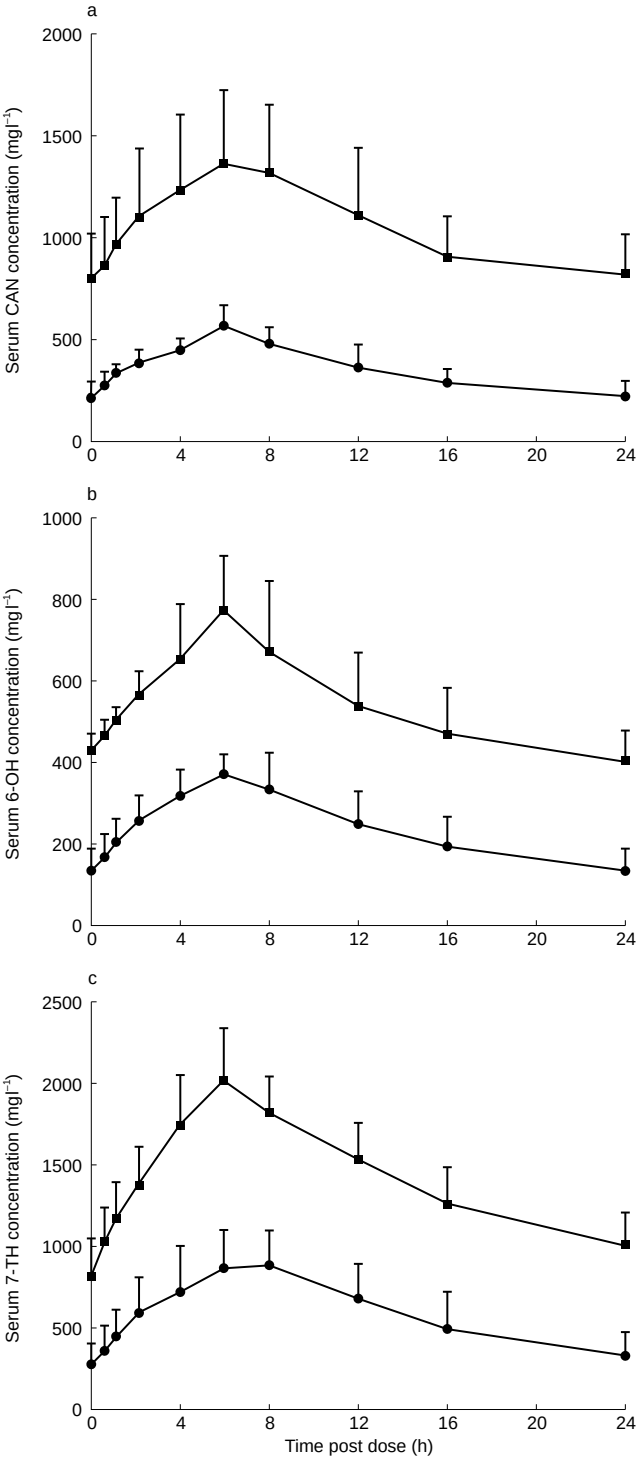

Figure 1 Steady state mean (s.d.) serum a) canrenone (CAN); b) 6β-hydroxy 7α-thiomethyl spironolactone (6-OH) and c) 7α-thiomethyl spironolactone (7-TH) concentrations following oral administration of the spironolactone: β-cyclodextrin complex capsules (■) and Aldactone tablets (●) 13 patients with chronic liver disease.

Discussion

The lack of a difference between the concentration at $t=0$ and $t=24$ indicates steady state conditions when each of the profiles was measured. Mean (s.d.) steady state half-lives of canrenone, 6β-hydroxy 7α-thiomethyl spironolactone and 7α-thiomethyl spironolactone of 16.5(6.3), 15.0 (4.0) and 13.8(6.5) h have been reported [14]. Other reports of the half-life for canrenone have ranged from 3.9 h [15] to 34.9 h [16]. However, these reports are from healthy individuals. Linear regression of all terminal profiles (using the four samples at $t=8$, 12, 16 and 24 h post dose) revealed half-lives of less than 30 h. These values are not reported because of the lack of data over a period greater than two half-lives. A washout period of 7 days should, therefore, have been adequate for the attainment of steady state conditions when the profiles were measured. Food has been shown to enhance the oral bioavailability of spironolactone [17, 18] and so all the spironolactone doses were given in the morning (shortly after waking) at least 1 h before breakfast.

It has been proposed that differences in the absorption of spironolactone tablets are due to the dissolution rate [19]. Since the spironolactone: β-cyclodextrin complex capsules demonstrated faster *in vitro* dissolution characteristics and

Table 2 Steady state pharmacokinetic data for canrenone (CAN), β-hydroxy 7α-thiomethyl spironolactone (6-OH) and 7α-thiomethyl spironolactone (7-TH) following oral administration of the spironolactone: β-cyclodextrin complex (SP-COMP) and Aldactone tablets (ALD). Values are mean (s.d.) except for t_{max} which are median (range).

	CAN		*6–0H*		*7-TH*	
	ALD	*SP-COMP*	*ALD*	*SP-COMP*	*ALD*	*SP-COMP*
AUC	8.15	24.35	5.68	13.01	13.79	34.15
($mg\,l^{-1}$ h)	(1.67)	(6.56)	(1.39)	(2.78)	(3.68)	(5.27)
C_{max}	0.54	1.36	0.39	0.78	0.99	2.03
($mg\,l^{-1}$)	(0.07)	(0.35)	(0.04)	(0.23)	(0.18)	(0.32)
t_{max}	6.0	6.0	6.0	6.0	8.0	6.0
(h)	(6.0–8.0)	(6.0–0.8)	(6.0–8.0)	(6.0–8.0)	(4.0–8.0)	(6.0–8.0)

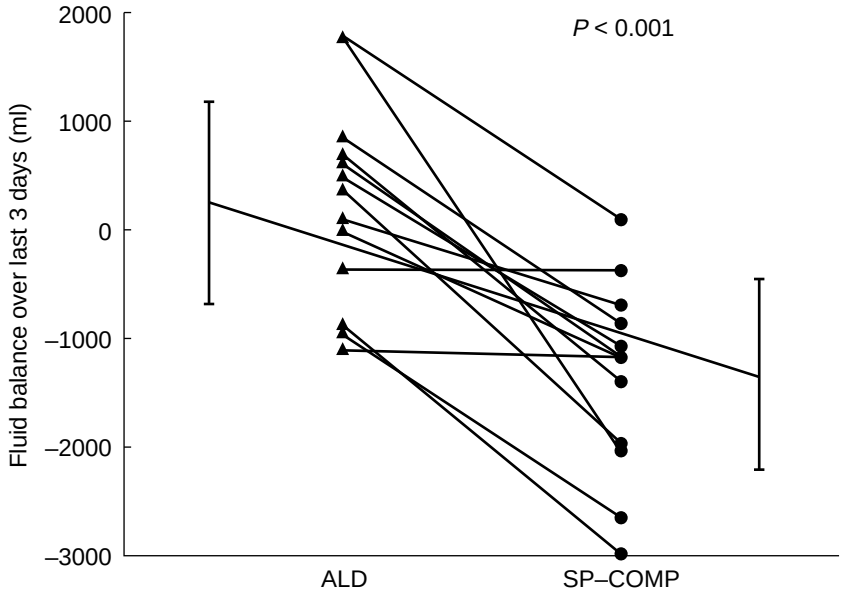

Figure 2 Mean (s.d.) and individual fluid balance over the last 3 days of the daily therapy with the spironolactone: β-cyclodextrin complex capsules and Aldactone tablets in 13 patients with chronic liver disease.

improved aqueous solubility then, as expected, the absorption was better. The area under the curve, used for these values, represents the fraction of the absorbed dose converted to each metabolite and their clearance. It is possible that in addition to inter-subject variability, due to differences in fractions metabolized and clearance, that there could be an intra subject variability related to the spironolactone dose swallowed or to improvements in clinical status. Clinical evaluation and the marked diuresis during the administration of the spironolactone: β-cyclodextrin therapy indicated that the health of these patients on this therapy improved. The decrease in ascitic fluid might have been associated with improved conditions for gastro-intestinal absorption and reduced hepatic congestion thereby improving metabolic processes. This could help to explain the difference in relative bioavailability for the three metabolites. Clinical status may also be the reason why the relative bioavailability for the spironolactone: β-cyclodextrin complex in these patients for the canrenone metabolites (301%) is greater than that (252%) which we have previously reported in healthy volunteers [12].

The clinical benefit gained from improving spironolactone bioavailability following administration of the spironolactone: β-cyclodextrin complex is demonstrated by the fluid balance and clinical examination. The overall mean (s.d.) fluid loss for the last 3 days of spironolactone: β-cyclodextrin therapy was −1370(860)ml whereas during Aldactone therapy there was a net gain of 228(930)ml for the same period. Values over the last 3 days have been used to allow sufficient time for all the spironolactone and its metabolites to be removed from the body from the previous formulation and because it takes 3–4 days for spironolactone to exert its diuretic effect. In clinical practice the use of a spironolactone: β-cyclodextrin formulation should allow a smaller dose of spironolactone to be given. The higher and more consistent absorption should enable patients to be managed more easily with a shorter period to stabilization and hence bed-stay may be decreased. However there is a formulation problem because the largest manageable capsules for patients contain the equivalent of only 25 mg spironolactone. Thus during this study one patient required 16 capsules to receive a 400 mg dose. Studies are in progress to formulate a higher dose tablet preparation which will shortly be used in further bioavailability studies.

We thank the staff of the Manufacturing and Quality Control Unit of the Pharmacy Department, St James's University Hospital, Leeds for the manufacture of spironolac-

tone : β-cyclodextrin capsules to approved specification. We also extend our thanks to the patients who volunteered to take part in the study and all the staff on wards 31 and 32 at St James's University Hospital, Leeds. Finally we are most grateful to Searle Pharmaceuticals, USA, for supplying all the spironolactone metabolites.

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(*Received 22 October 1996, accepted 11 March 1997*)