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Water-soluble β-Cyclodextrins in Paediatric Oral Solutions of Spironolactone: Preclinical Evaluation of Spironolactone Bioavailability from Solutions of β-Cyclodextrin Derivatives in Rats

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Abstract

Water-soluble derivatives of β-cyclodextrin have been considered for solubilization of spironolactone in the formulation of a safe liquid preparation for premature infants. The oral absorption of spironolactone was studied in rats to evaluate the need to adjust spironolactone dosage in prospective clinical studies. Spironolactone was administered in solutions of sulphobutyl ether β-cyclodextrin (SBE7) or dimethyl-β-cyclodextrin (DM-β-CyD) and also as spironolactone-containing powder papers (reference preparation). Spironolactone in SBE7 solution was administered intravenously to assess the extent of intestinal absorption from the different formulations.

Spironolactone and the metabolites 7α-thiospirolactone, 7α-thiomethylspirolactone and canrenone were determined in rat serum after intravenous administration of spironolactone. Half-lives for spironolactone, 7α-thiomethylspirolactone and canrenone were 0·72 ± 0·17, 1·5 ± 0·3 and 2·2 ± 0·3 h, respectively. Although, according to C_{max} values, 7α-thiomethylspirolactone was the major serum metabolite in rats, higher AUC (area under the serum concentration–time curve) values were obtained for canrenone. After oral administration of spironolactone the bioavailabilities evaluated from the AUC values of 7α-thiomethylspirolactone were 27·5 ± 9·3%, 81·3 ± 28·8% and 82·8 ± 28·6% for powder papers, DM-β-CyD and SBE7 solutions, respectively.

The oral absorption of spironolactone by rats was better after administration of spironolactone in SBE7 and DM-β-CyD solutions than after administration as powder papers. Both cyclodextrin formulations enhanced spironolactone bioavailability to a similar extent despite some deacetylation of spironolactone in the presence of SBE7. A reduction of spironolactone dosage would be recommended during clinical studies with premature infants. These results indicate that SBE7 could be a safe and suitable excipient for the solubilization of spironolactone in paediatric formulations.

Spironolactone, a competitive aldosterone antagonist, is used as a potassium-sparing diuretic in premature infants to improve lung function by reduction of pulmonary oedema (Albersheim et al 1989; Kao et al 1994). Liquid formulations are preferable for oral medication in neonates, as administration is achieved through a thin nasogastric tube. High amounts of co-solvents (Pramar et al 1992) or high osmolality syrups as suspending agents (Committee on Extemporaneous Formulations 1987; Mathur & Wickman 1989) have been used in liquid formulations to overcome the poor aqueous solubility of spironolactone. Both approaches carry potential risks for neonatal patients, however, either through the metabolic immaturity of the neonate or local intestinal effects (Leff & Roberts 1987; Tötterman et al 1994). In the absence of suitable liquid preparations of spironolactone, powder papers prepared in lactose from commercial spironolactone tablets are presently used at the

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Children's Hospital, Helsinki University Central Hospital. Administration of the powders mixed with only a small volume of water (1–2 mL) frequently results in loss of a variable part of the dose and obstruction of the feeding tube. Water-soluble derivatives of β-cyclodextrin have been considered for solubilization of spironolactone to formulate a safe liquid preparation which would also provide consistent delivery of spironolactone.

Cyclodextrins are cyclic oligosaccharides that improve the solubility of lipophilic drugs by molecular encapsulation, i.e. by inclusion of the drug into the hydrophobic cavity of the cyclodextrin (Szejtli 1988). β-Cyclodextrin has been used to increase the solubility of spironolactone and thereby its oral bioavailability from solid dosage forms administered to dogs, rats and man (Seo et al 1983; Debruères et al 1985; Vila-Jato et al 1986; Yusuff et al 1991) and there is evidence of improved clinical response (Abosehmah-Albidy et al 1997). The use of β-cyclodextrin for formulation of a spironolactone solution is hampered by the low solubility of the cyclodextrin itself and of its drug complexes (Seo et al 1983; Debruères et al 1985; Yusuff & York 1991). Dimethyl-β-cyclodextrin (DM-β-CyD) (Uekama 1985) and hydroxypropyl-β-cyclodextrin (HP-β-CyD) (Pitha et al 1986), themselves highly soluble, have been reported to solubilize large amounts of spironolactone. Evidence of the deacetylation of spironolactone on complexation with the parent cyclodextrin (Szejtli 1988; Wouessidjewe et al 1989) further supported the use of modified β-cyclodextrins.

Previous work has shown that HP-β-CyD and sulphobutyl ether β-cyclodextrin (SBE7) have little effect on intestinal epithelial integrity and appear to be safe additives with respect of their local effects in the intestine (Tötterman et al 1997). Dose-dependent cytotoxicity has, however, been observed for DM-β-CyD. A study of spironolactone solubilization and stability revealed that deacetylation of spironolactone in the presence of HP-β-CyD and SBE7 was temperature-dependent, with slower degradation in solutions of SBE7 than in HP-β-CyD (Kaukonen et al 1997). No degradation occurred in the presence of DM-β-CyD. The conclusion was that if the solutions were prepared and stored at 6°C or below SBE7 could be considered for solubilization of spironolactone in paediatric enteral solutions. DM-β-CyD, despite the superior stability of spironolactone, could not be considered because of its cytotoxic effects on intestinal epithelial cells.

Our main objective in this study was to compare the oral absorption of spironolactone in the rat from SBE7 and DM-β-CyD solutions, relative to that from spironolactone-containing powder papers, and thus to evaluate the possible need to adjust the dosage of spironolactone in prospective clinical studies. DM-β-CyD solutions were included in the study to evaluate the possible effects of spironolactone degradation in SBE7 solutions. The areas under the serum concentration–time curves (AUC) of the two main active metabolites 7α-thiomethylspirolactone and canrenone (Overdiek et al 1985; Overdiek & Merkus 1987) were used as measures of spironolactone bioavailability. Spironolactone in SBE7 solution was administered intravenously to assess the extent of intestinal absorption from the different formulations. Intravenous administration also provided new information on the pharmacokinetics of spironolactone in rats.

Materials and Methods

Chemicals

HPLC-grade methanol and acetonitrile (Rathburn Chemicals) were used for preparation of samples and for chromatography. Deionized water was purified by means of a Milli-Q Plus system (Millipore, Bedford, MA). Spironolactone, 7α-thiospirolactone, 7α-thiomethylspirolactone and 6β-hydroxy-7α-thiomethylspirolactone were kindly provided by Searle (Skokie, IL) and canrenone by Searle Pharmaceuticals (Morpeth, UK) (Figure 1). Heptakis 2,6-di-*O*-methyl-β-cyclodextrin (DM-β-CyD; degree of substitution 14; Figure 2) and the internal standard methyltestosterone were purchased from Sigma (St Louis, MO). Sodium sulphobutyl ether β-cyclodextrin (SBE7, Captisol; degree of substitution 7; Figure 2) was kindly donated by CyDex (Overland Park, KS).

Animals and treatment

Experiments were performed with adult male Wistar rats, 611 ± 65 g, 36 ± 5 weeks. Rats were deprived of food for 20–24 h before drug administration. During fasting the rats were kept in cages with wide screen bottoms to prevent coprophagy. Food was provided 4 h after drug administration; tap water was always freely available. The intra-arterial catheter (PE-50 polyethylene; o.d. 0·96 mm) needed for collection of blood samples was inserted into the carotid artery under anaesthesia (intraperitoneal injection of xylazine, 15 mg kg^{-1}, and ketamine, 85 mg kg^{-1}) the day before the experiment (Lennernäs & Regårdh 1993). Rats administered intravenous doses also had their jugular vein similarly catheterized. The heparinized catheters were passed under the skin

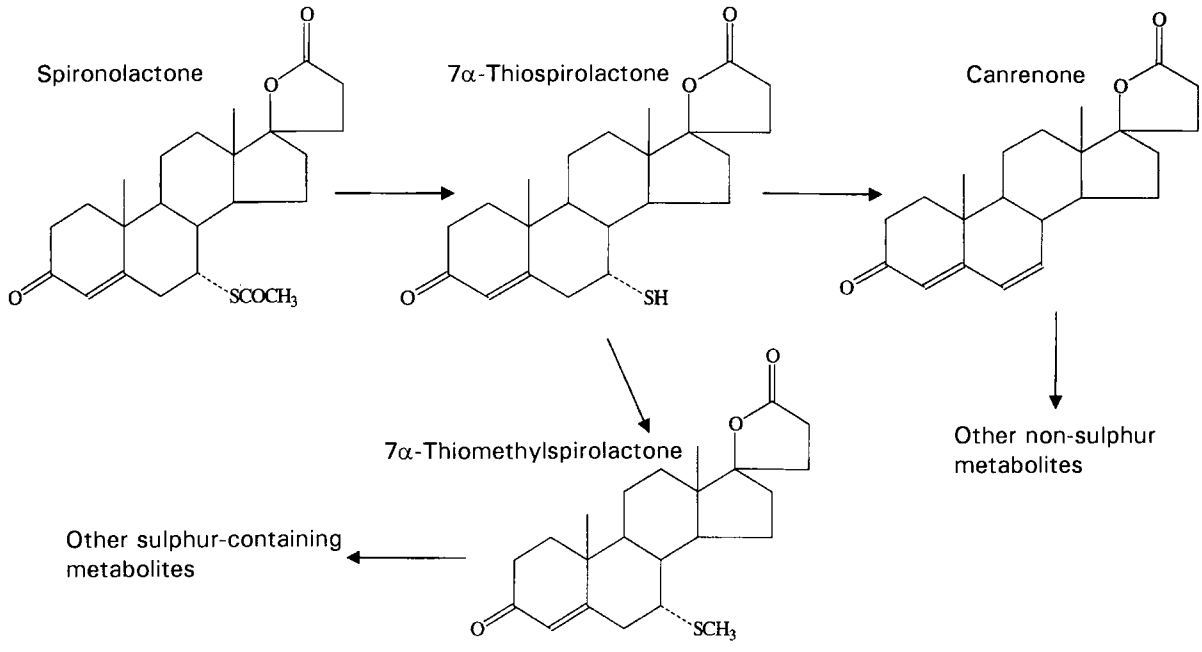

Figure 1. Proposed metabolic pathway of spironolactone.

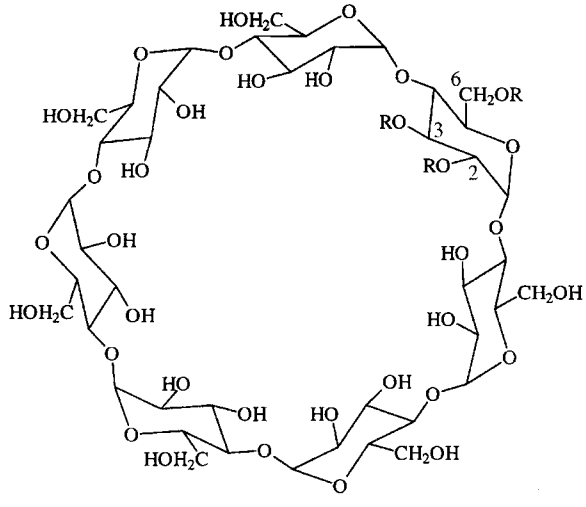

Figure 2. Molecular structures of modified β-cyclodextrins. Sulphobutyl ether β-cyclodextrin is variably substituted at positions 2, 3 or 6 with R = $-CH_2CH_2CH_2CH_2SO_3^-Na^+$. Dimethyl-β-cyclodextrin is specifically substituted at positions 2 and 6 with R = $-CH_3$.

and exteriorized at the back of the neck. A polypropene hat was sown on to the rats' necks to shield the catheters.

Preparation of formulations

Spironolactone-containing powder papers, which are the formulation currently used at the Children's Hospital, were used as oral reference formulation. The powder papers were prepared at the Hospital Pharmacy, Helsinki University Central Hospital, by first grinding 50 mg Aldactone tablets (Searle), which were then diluted with lactose to a spironolactone concentration of 5 mg/100 mg powder. Before administration distilled water (1 mL/100 mg powder) was added to the powder and the mixture thoroughly shaken.

Oral solutions of spironolactone (5 mg mL^{-1}) were prepared in autoclaved 48 mM solutions of DM-β-CyD and SBE7 (molar ratio of spironolactone to cyclodextrin, 1 : 4). Solutions (5 mg mL^{-1}) for intravenous dosing of spironolactone were prepared in autoclaved 48 mM solutions of SBE7. The temperature of the SBE7 solution was 6°C when spironolactone was added and the solutions were kept on ice during solubilization to minimize the degradation of spironolactone (Kaukonen et al 1997). The SBE7 solutions were administered within 29 ± 10 min of addition of the spironolactone. DM-β-CyD solutions were prepared at room temperature as spironolactone has been found to be stable in the presence of DM-β-CyD (Kaukonen et al 1997).

Oral and intravenous dosing

Preparations containing 5 mg mL^{-1} spironolactone were administered orally by gavage at a dose of 50 mg kg^{-1}. Blood samples were withdrawn before dosing (control) and 0·5, 1, 1·5, 2, 3, 4, 6, 8, 10, 12 and 14 h after dosing. The samples were withdrawn into vials for serum collection (Microvette CB 1000 S; Sahrstedt, Nümbrecht, Germany), centrifuged at 6000 *g* for 2 min (Eppendorf, 5415 C) and the serum stored at −20°C. A maximum sample volume of 1 mL kg^{-1} was collected from individual rats at each time-point. After collection of the last

sample the rats were immediately killed with carbon dioxide.

Preparations containing 5 mg mL^{-1} spironolactone were administered intravenously as bolus injections into the jugular vein over 1·5 min at a dose of 20 mg kg^{-1}. The catheter was then flushed with 0·3 mL saline. Blood samples were withdrawn before dosing (control) and 5, 10 and 15 min and 0·5, 1, 2, 3, 4, 6, 8 and 12 h after dosing; they were treated as above.

Analysis of serum samples

Serum concentrations of spironolactone and its metabolites were determine by means of an HPLC-method developed for this purpose (Kaukonen et al 1998). HPLC was performed with a Waters 510 solvent-delivery system, in-line degasser, 717 Plus autosampler and a 490 programable multiple wavelength UV-vis detector. Data and system management was handled by a Millennium 2010 Chromatography Manager. A LichroCart steel cartridge column (Merck, Darmstadt, Germany) packed with Spherisorb ODS-2 (5 μm, 250 mm × 4 mm) was used with an integrated pre-column (Spherisorb, 4 mm × 4 mm) at ambient temperature. The mobile-phase flow-rate was 1·0 mL min^{-1}; detection was performed at 238 nm for spironolactone, 7α-thiospirolactone, 7α-thiomethylspirolactone and 6β-hydroxy-7α-thiomethylspirolactone and at 280 nm for canrenone. The mobile-phase consisted of 67% methanol in water. Stock solutions and dilutions of standards were prepared in pure acetonitrile with four replicates of six concentrations (n = 24). Methyltestosterone (0·5 mg mL^{-1}) was used as internal standard. Samples were prepared by adding standard solution (100 μL) to blank rat serum (100 μL). The samples were vortex-mixed for 15 s before centrifugation (FP-510, Labsystem, Finland) at 5000 rev min^{-1} for 3 min. The supernatant was placed in autosampler vials and 100-μL samples were injected.

Linear calibration plots for spironolactone, 7α-thiospirolactone, 7α-thiomethylspirolactone, 6β-hydroxy-7α-thiomethylspirolactone and canrenone were obtained over the ranges 100–700, 150–550, 100–1800, 150–600 and 50–1300 ng mL^{-1}, respectively. Regression coefficients (r) were 0·998, 0·992, 0·997, 0·992 and 0·998, respectively. Recoveries of the compounds, determined during method development and validation, were in the range 93·1–117·3%. Mean accuracy ranged between −11·8% and 13·4% (Kaukonen et al 1998).

For analysis of samples, acetonitrile containing the internal standard (100 μL) was added to serum (100 μL) and the samples were treated as above.

Calculation of pharmacokinetic parameters

The area under the serum concentration–time curve, AUC, was calculated by the trapezoidal rule for intravenous and oral doses. Elimination coefficients (k_{el}) and half-lives ($t_{1/2}$) were determined by linear regression analysis of the log-linear terminal phase of the serum concentration–time curves obtained from intravenously treated rats. The elimination coefficients were used to calculate the remaining area to obtain $AUC_{0-\infty}$ values for spironolactone, 7α-thiomethylspirolactone and canrenone in intravenously treated rats. The AUC_{0-14} values were calculated after oral administration because elimination coefficients could not be properly determined for all the rats. As no AUC for spironolactone could be determined after oral administration the AUC values of the main active metabolites 7α-thiomethylspirolactone and canrenone were used to assess spironolactone bioavailability from oral preparations. The fraction absorbed with respect to metabolites (F_{metab}) was calculated for 7α-thiomethylspirolactone and canrenone by use of the equation:

$$F_{metab}(\%) = \frac{(D_{i.v.} \times AUC_{0-14\ oral})}{(D_{oral} \times AUC_{0-\infty i.v.})} \times 100 \quad (1)$$

where $AUC_{0-\infty\ i.v.}$ is the mean obtained from intravenously treated rats and $D_{i.v.}$ and D_{oral} are the intravenous and oral doses of spironolactone (mg kg^{-1}), respectively. Relative bioavailabilities of spironolactone from DM-β-CyD and SBE7 solutions compared with powder papers were calculated from AUC_{0-14} values of 7α-thiomethylspirolactone and canrenone. Peak serum concentrations, C_{max}, were taken from the highest measured serum concentration; t_{max} is the time corresponding to this value.

Statistics

All results are presented as the mean and the standard deviation (s.d.) except in figures, where the mean and the standard error of the mean (s.e.m.) are used. One-factor analysis of variance was used for statistical analysis between formulations; this was followed by the Tukey highly significant difference test for pair-wise comparisons.

Results and Discussion

Intravenous administration

Spironolactone, 7α-thiospirolactone, 7α-thiomethylspirolactone and canrenone were determined in rat serum samples after intravenous administration of spironolactone (20 mg kg^{-1}) (Figure 3). Half-lives determined from log-linear concentra-

tion–time data for spironolactone, 7α-thiomethylspirolactone and canrenone were, respectively, 0·72 ± 0·17, 1·5 ± 0·3 and 2·2 ± 0·3 h (Table 1). Comparable serum concentrations of spironolactone and 7α-thiospirolactone were determined 5 min after dosing (6425 ± 1087 ng mL^{-1} and 7720 ± 2248 ng mL^{-1}). The half-life of 7α-thiospirolactone, 7·9 ± 2·3 min, seems very short but should be taken as an approximation because of the low number of data points during the elimination phase. According to C_{max} values 7α-thiomethylspirolactone (C_{max} 2105 ± 64 ng mL^{-1}) was the major serum metabolite in all rats; the next most important metabolite was canrenone (C_{max} 780 ± 100 ng mL^{-1}). This is in accord with previous results in man (Overdiek et al 1985; Gardiner et al 1989) and guinea-pig (Sherry et al 1986; Los et al 1993). In contrast with results from man (Overdiek et al 1985; Gardiner et al 1989), in this study the $AUC_{0-\infty}$ value for canrenone was higher than that for 7α-thiomethylspirolactone (Table 1). The sulphur-containing metabolites have not previously been determined in rat serum, although Cook et al (1988) have reported that spironolactone metabolism seemed to predominantly follow a pathway by which sulphur was retained in the molecule. However, their study focused on the separation of canrenone and canrenone-related metabolites.

In man spironolactone, but no 7α-thiospirolactone, has been determined in serum after single or multiple oral doses of spironolactone (Overdiek et al 1985; Gardiner et al 1989). In guinea-pig plasma 7α-thiospirolactone and trace amounts of spironolactone have been measured 4 h after intraperitoneal administration of spironolactone (Los et al 1993). The high levels of 7α-thiospirolactone in this study could partly be explained by species differences in enzymatic activity and disposition (Karim et al 1976; Los et al 1993), further accentuated by the different routes of administration. In addition, 7α-thiospirolactone is also formed by deacetylation of spironolactone in the presence of SBE7 (Kaukonen et al 1997) and is therefore present in the formulation at the time of administration. Because of the method of preparation, degradation of spironolactone in the formulation is limited to a maximum of 5% (Kaukonen et al 1997). As a consequence of elimination faster than that of spironolactone the decline of 7α-thiospirolactone concentration should parallel that of spironolactone. Because parallel decline is not apparent it seems, however, that a sufficient amount of the administered spironolactone has been converted to 7α-thiospirolactone to show, temporarily, the true elimination of the metabolite. These results would imply continuing deacetylation of spironolactone by SBE7 and, hence, incomplete dissociation during early circulation.

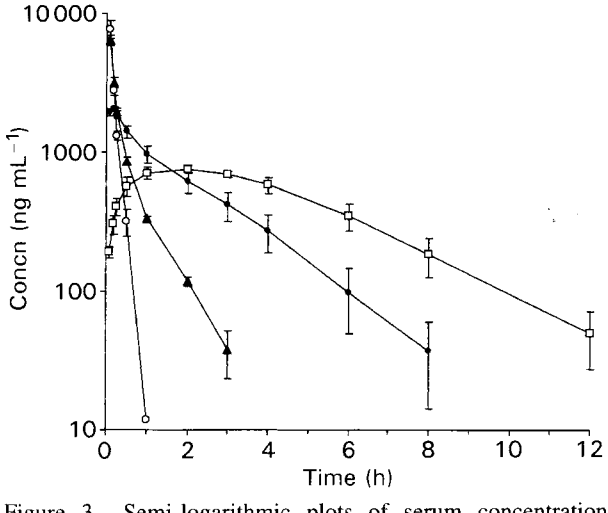

Figure 3. Semi-logarithmic plots of serum concentration against time for spironolactone (▲), 7α-thiospirolactone (○), 7α-thiomethylspirolactone (●) and canrenone (□) in rats (mean ± s.e.m., n = 4) after intravenous administration of 20 mg kg^{-1} spironolactone in SBE7 solution.

Dissociation of drug–cyclodextrin complexes is usually achieved very effectively by dilution when relatively weakly complexed drugs ($K_{1:1} < 10\,000$) are administered (Szejtli 1988; Stella & Rajewski 1997). A maximum dilution of 1 : 75 upon intravenous administration of this formulation could be achieved according to the inulin-like pharmacokinetic behaviour of SBE7 (Rajewski et al 1995; Harding et al 1997). The high stability constant of 18 200 M^{-1} determined for spironolactone and SBE7 complexes (Kaukonen et al 1997), suggests initially insufficient dilution especially as the formulation contains a molar excess of cyclodextrin over spironolactone (spironolactone/SBE7 molar ratio 1 : 4) (Szejtli 1988). Competitive displacement of spironolactone by lipophilic plasma components and the high binding of spironolactone to plasma proteins (Frijlink et al 1991) should nevertheless induce complete dissociation.

In conclusion, SBE7 in the formulation altered spironolactone pharmacokinetics to some extent, initially by reducing spironolactone concentrations and thereby reducing the $AUC_{0-\infty}$ values obtained from serum concentration–time profiles (Table 1). As the concentration of 7α-thiospirolactone, the first step in the metabolic pathway (Sadée et al 1974; LaCagnin et al 1987), was increased effects on consecutive metabolites are difficult to distinguish. The current results give, despite the influence of SBE7, valuable information on spironolactone metabolism because recent analytical techniques have not previously been used for

Table 1. Elimination coefficient, half-life and area under the serum concentration–time curve ($AUC_{0-\infty}$) for spironolactone, 7α-thiomethylspirolactone and canrenone.

	Elimination coefficient (h^{-1}) (n = 5)	Half-life (h) (n = 5)	$AUC_{0-\infty}$ (ng h mL^{-1}) (n = 4)
Spironolactone	0·99 ± 0·18	0·72 ± 0·17	1628 ± 129
7α-Thiomethylspirolactone	0·47 ± 0·09	1·5 ± 0·3	3711 ± 1261
Canrenone	0·32 ± 0·06	2·2 ± 0·3	4700 ± 994

Values are mean ± s.d. Spironolactone was intravenously administered to rats in 48 mM SBE7 solution at 20 mg kg^{-1}.

quantitative study of spironolactone pharmacokinetics in rats. Furthermore, the $AUC_{0-\infty}$ values of 7α-thiomethylspirolactone and canrenone are measures of spironolactone availability which can be used in the assessment of oral bioavailability.

Oral administration of spironolactone

A softening effect on the stools could be observed during the last 4 h of sampling in many rats receiving cyclodextrin solutions and powder papers. This is an effect common to diets containing large amounts of poorly digested carbohydrates, as is observed for lactose in rats (Schulze & Zunft 1991; Thompson 1997). The oral doses of SBE7 and DM-β-CyD, 1040 and 640 mg kg^{-1}, were high because of the high dose of spironolactone in rats.

7α-Thiomethylspirolactone and canrenone were determined in serum samples of all rats after oral administration of spironolactone at 50 mg kg^{-1} as powder papers or cyclodextrin solutions (Figures 4 and 5). Samples collected 0·5–2 h after dosing contained small amounts of spironolactone or 7α-thiospirolactone, or both, in two, three and four of the rats administered spironolactone in powder papers, DM-β-CyD and SBE7 solutions, respectively. Administration of spironolactone in SBE7 and DM-β-CyD solutions resulted in higher values of C_{max} and AUC_{0-14} for both 7α-thiomethylspirolactone and canrenone than did administration of powder papers (Figures 4, 5; Table 2). The C_{max} values of 7α-thiomethylspirolactone were higher than those of canrenone when cyclodextrin formulations were used, whereas the opposite was true for the powder papers (Table 2). As was found for intravenous administration, each of the oral formulations led to higher mean AUC_{0-14} values for canrenone than for 7α-thiomethylspirolactone. The sampling period of 14 h resulted in truncated concentration–time profiles of canrenone because of its slower elimination (Figures 4, 5; Table 1), whereas the more complete profiles of 7α-thiomethylspirolactone led to AUC_{0-14} values that should closely reflect their respective $AUC_{0-\infty}$ values. Discussion of oral bioavailability is, therefore, mainly based on the pharmacokinetic parameters obtained for 7α-thiomethylspirolactone.

Oral bioavailability of spironolactone

The oral bioavailability of spironolactone (F_{metab}), determined in respect of AUC values of 7α-thiomethylspirolactone, was 27·5 ± 9·3%, 81·3 ± 28·8% and 82·8 ± 28·6% from powder papers, DM-β-CyD and SBE7 solutions, respectively. Enhanced bioavailability from formulations containing cyclodextrins than from powder papers was also evidenced by the high relative bioavailability of spironolactone from DM-β-CyD (296 ± 105%) and SBE7 solutions (302 ± 104%). Here, as in previous studies (Seo et al 1983; Debruères et al 1985; Vila-Jato et al 1986; Yusuff et al 1991), the enhanced bioavailability of spironolactone could probably be ascribed to the increased solubility of the drug and the thereby increased absorption. Slightly faster absorption of spironolactone seems to occur from the SBE7 solution in comparison with the DM-β-

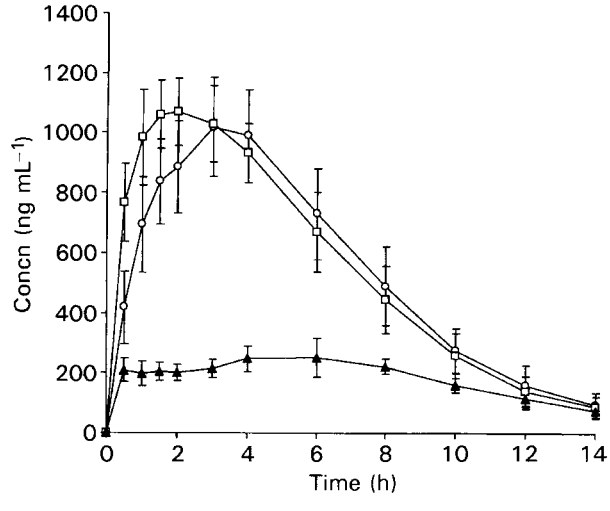

Figure 4. Plots of serum concentration of 7α-thiomethylspirolactone against time in rats (mean ± s.e.m., n = 5) after oral administration of 50 mg kg^{-1} spironolactone. ▲ Powder papers; □ solutions of 48 mM SBE7; ○ solutions of 48 mM DM-β-CyD.

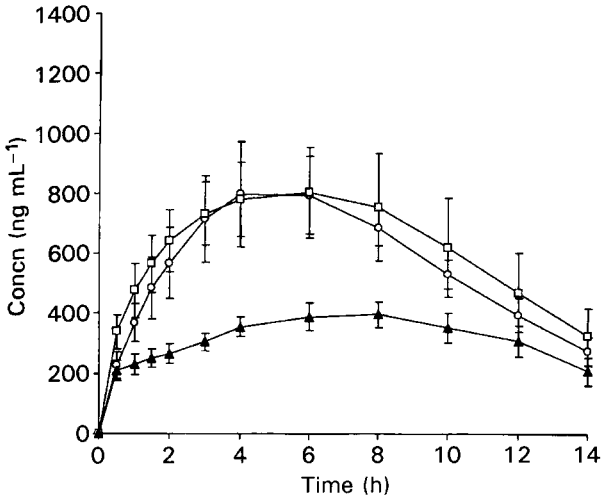

Figure 5. Plots of serum concentration of canrenone against time in rats (mean ± s.e.m., n = 5) after oral administration of 50 mg kg^{-1} spironolactone. ▲ Powder papers; □ solutions of 48 mM SBE7; ○ solutions of 48 mM DM-β-CyD.

CyD solution (Figure 4, Table 2), possibly reflecting the differences in stability constants of spironolactone complexes (Szejtli 1988). $K_{1:1}$ values of 50 200 M^{-1} and 18 200 M^{-1} have been determined for spironolactone–DM-β-CyD and spironolactone–SBE7 complexes, respectively (Kaukonen et al 1997), again raising the question of potential incomplete dissociation, especially as the solutions contained molar excesses of cyclodextrins. The effect of dilution after oral administration is smaller than that after intravenous administration, but competitive displacement should strongly contribute to dissociation. Prolonged absorption could be surmised, with part of the spironolactone dose dissociating slowly during passage through the intestines. This possibility is supported by the longer elimination half-lives of 7α-thiomethylspirolactone in rats after oral rather than intravenous administration of cyclodextrin solutions (2·26 ± 0·47 h compared with 1·5 ± 0·3 h). More evidence could have been obtained by administering solutions with lower molar ratios of spironolactone to DM-β-CyD; this has a higher stability constant without the complications caused by spironolactone degradation.

According to the current results the degradation of spironolactone in the presence of SBE7 does not seem to affect the bioavailability of spironolactone evidenced by the similar AUC_{0-14} values of the two cyclodextrin formulations (Table 2). Oral administration of solid spironolactone–HP-β-CyD complexes, likely to contain most of the dose as deacetylated spironolactone (Kaukonen et al 1997), has been reported in dogs to lead to AUC values for canrenone 3·6 times higher than after administration of spironolactone alone (Soliman et al 1997). Similarly, increased oral biovailability and improved clinical response have been observed with solid spironolactone–β-CyD complexes in man (Yusuff et al 1991; Abosehmah-Albidy et al 1997). According to these results the low therapeutic effect of orally administered 7α-thiospirolactone (McInnes et al 1980) has correctly been attributed to the poor bioavailability of this compound (Overdiek et al 1985). This suggests that 7α-thio-spirolactone, which is the metabolic precursor to 7α-thiomethylspirolactone and canrenone, could indeed be credited with therapeutic effects upon oral administration (Overdiek et al 1985; Kaukonen et al 1997).

Clinical relevance and implications

In premature infants the currently recommended dosage of spironolactone is 2–4 mg kg^{-1} day^{-1} administered as powder papers. The aim was therefore to formulate a solution containing 3 mg mL^{-1} for easy administration and low water

Table 2. Absorption characteristics of the spironolactone metabolites 7α-thiomethylspirolactone and canrenone in the rat after oral administration of 50 mg kg^{-1} spironolactone.

Preparation	C_{max}* (ng mL^{-1})	t_{max} (h)	AUC_{0-14}† (ng h mL^{-1})	Fraction absorbed (%)
7α-Thiomethylspirolactone				
Powder paper	296 ± 120	4·6 ± 2·6	2546 ± 865	27·5 ± 9·3
DM-β-CyD solution	1088 ± 366‡	3·8 ± 1·3	7545 ± 2668¶	81·3 ± 28·8
SBE7 solution	1191 ± 281‡	2·3 ± 1·0	7681 ± 2656‡	82·8 ± 28·6
Canrenone				
Powder paper	420 ± 92	7·2 ± 1·1	4414 ± 1062	37·6 ± 9·0
DM-β-CyD solution	873 ± 332	6·0 ± 2·0	7959 ± 1855	67·7 ± 15·8
SBE7 solution	842 ± 312	5·4 ± 2·0	8654 ± 3990	73·7 ± 34·0

Values are mean ± s.d. (n = 5). *Significant difference between formulations for values for both 7α-thiomethylspirolactone ($P < 0·001$) and canrenone ($P < 0·05$) (analysis of variance). †Significant difference between formulations for values for 7α-thiomethylspirolactone ($P < 0·01$) (analysis of variance). ‡$P < 0·01$, significantly different from result for powder paper (Tukey test). ¶$P < 0·05$, significantly different from result for powder paper (Tukey test).

load. The increased oral bioavailability observed in this work implies that the spironolactone dose could be reduced to 1–2 mg kg^{-1} day^{-1}, i.e. a solution containing 1–2 mg mL^{-1} of spironolactone. With the molar ratio of spironolactone to cyclodextrin kept at 1 : 4, a prerequisite for rapid preparation of the solution (Kaukonen et al 1997), the dose of SBE7 would be as low as 21–42 mg kg^{-1}. This amount of SBE7, although likely to be practically non-digestible (Thompson 1997), should not present a problem, nor should the small amount of sodium ions originating from the sulphated substituents of SBE7 (Kaukonen et al 1997). Possible effects in rats as a result of incomplete dissociation of the highly bound spironolactone–SBE7 complex after oral administration should be reduced in premature infants because of the smaller dose of spironolactone and, correspondingly, the smaller amount of cyclodextrin. This should also ensure consistent absorption of spironolactone. In comparison with powder papers, administration of the solution should provide consistent delivery of the specified dose of spironolactone without random losses of the dose or blockage of the nasogastric tube. Furthermore, replacement of powder papers with cyclodextrin solutions would reduce the daily dose of lactose, to the benefit of those premature infants suffering from low lactase activity (MacClean & Fink 1980).

In conclusion, the oral bioavailability of spironolactone in rats was higher after administration in SBE7 and DM-β-CyD solutions than after use of powder papers. The increase in AUC values of spironolactone metabolites, probably resulting from increased absorption of spironolactone or its metabolites, or both, was similar for both formulations containing cyclodextrin, despite some deacetylation of spironolactone in the presence of SBE7. A reduction of spironolactone dosage is recommended when clinical studies are performed with premature infants. These and previous results indicate that SBE7 could be a safe and suitable excipient for the solubilization of spironolactone in paediatric formulations, assuming absorption characteristics and pharmacokinetics are confirmed to be similar for infants and adults.

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