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Nanosuspension Formulations for Low-Soluble Drugs: Pharmacokinetic Evaluation Using Spironolactone as Model Compound

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ABSTRACT Various particle sizes of spironolactone as a model low solubility drug were formulated to yield micro-and nanosuspensions of the type solid lipid nanoparticles and DissoCubes®. Seven oral and one i.v. formulations were tested in an in vivo pharmacokinetic study in rats with the aim of characterizing the bioavailability of spironolactone on the basis of its metabolites canrenone and 7-α-thiomethylspirolactone. In addition, a dose escalation study was carried out using nonmicronized spironolactone suspension as well as a nanosuspension type DissoCubes®. On the basis of AUC as well as C_{max} ratios, three groups of formulations were distinguished. The biggest improvement was seen with a solid lipid nanoparticle formulation yielding a 5.7-fold increase in AUC for canrenone and a similar improvement based on the C_{max} metric, followed by a group of three formulations containing nanosized, micronized, and coarse drug material and surfactant. The DissoCubes® nanosuspension yielded highly significant improvements in bioavailability averaging 3.3-fold in AUC and 3.0-fold in terms of C_{max} for canrenone. The third class encompasses all other formulations, which showed very little to no improvement in bioavailability. The results show that the particle size minimization was not the major determining factor in the bioavailability improvement. Rather, the type of surfactant used as stabilizer in the formulations was of greater importance. Improvement in drug solubility in the intestine as well as in dissolution rate of spironolactone are the most likely mechanisms responsible for the observed effect, although additional mechanisms such as permeability enhancement may also be involved.

KEYWORDS Absorption, Bioavailability, Solubility, Particle size, Oral drug delivery

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INTRODUCTION

Biopharmaceutically acceptable formulations for drugs with low solubility in aqueous solvents are a challenge since slow and erratic dissolution is preventing the rapid and complete absorption of these compounds from the gastrointestinal tract. Nanosuspensions may show improved dissolution and solubility and, therefore, may represent an alternative to existing drug delivery technologies for these type of compounds (Hu et al., 2004). In the present study, the performance of nanosuspensions as potential vehicles for low soluble compounds is investigated. As model compound spironolactone was chosen. Spironolactone has a solubility of merely 2.8 mg/100 mL at 25°C in water (Overdiek & Merkus, 1987). It is extensively metabolized to 7-α-thiospirolactone, canrenone, 7-α-thiomethylspirolactone, and 6-β-hydroxy-7-α-thiomethylspirolactone. In man, 7-α-thiomethylspirolactone has been found to be the main and active metabolite, followed by canrenone (Overdiek & Merkus, 1986). Also in rats, highest blood concentrations were found for 7-α-thiomethylspirolactone, followed by canrenone. The lowest concentrations were found for 6-β-hydroxy-7-α-thiomethylspirolactone (Kaukonen et al., 1998).

The purpose of this study was 1) to evaluate the bioavailability of spironolactone formulated as solid lipid nanoparticles and as DissoCubes®, in comparison with other conventional formulations, 2) to investigate the influence of surfactant added to suspensions of micronized and coarse spironolactone on its bioavailability and 3) to investigate the dose linearity of the pharmacokinetics following administration of selected oral spironolactone formulations. For that purpose, the concentration-time-course of the parent compound and its metabolites was monitored in vivo in rats after intravenous and oral dosing, respectively. Bioavailability estimates were obtained following noncompartmental pharmacokinetic analysis.

MATERIALS AND METHODS

Formulations

A. Spironolactone nanosuspension type DissoCubes® consisted of 10% spironolactone, 0.5% dioctylsulfosuccinate, and 89.5% demineralized water and was prepared by incorporating progressively the surfactant solution to the active drug and then mixed for 1 minute at 11,000 rpm using a high shear dispenser (Polytron, Kinematika, Luzern, Switzerland). The suspension was then prehomogenized for 3 minutes at 500 bars and thereafter homogenized at 1500 bars for 300 minutes using a high pressure homogenizer (Avestin Emulsiflex C50, Avestin Inc., Ottawa, Canada). The high pressure homogenization step was carried out under controlled temperature allowing a maximum temperature of 20°C. The mean particle size of the spironolactone material was 400 nm as determined by photon correlation spectroscopy. Laser diffraction analysis yielded by volume 10% $<$0.4 μm, 50% $<$0.54 μm, and 90% $<$0.67 μm.
B. Spironolactone suspension (10% nonmicronized spironolactone in sirupus simplex DAB 10). Preparation was in accordance with a previously published method (Mathur & Wickman, 1989; Nahata et al., 1993). Particle size analysis of the nonmicronized material by laser diffraction analysis yielded by volume 10% $<$1.096 μm, 50% $<$8.012 μm, and 90% $<$15.04 μm.
C. Spironolactone solution consisted of 0.2% spironolactone in a cosolvent system of 10% EtOH (v/v), 30% polyethylene glycol 400 (v/v), 10% propylene glycol (v/v) in phosphate buffer (Pramar et al., 1992). This formulation was used for intravenous administration.
D. Spironolactone microsuspension consisted of 10% micronized spironolactone in sirupus simplex DAB 10. Particle size analysis of the micronized spironolactone yielded by volume 10% $<$0.615 μm, 50% $<$1.834 μm, and 90% $<$4.151 μm. The preparation was similar to formulation B.
E. Spironolactone microsuspension with surfactant consisted of 10% micronized spironolactone, 0.5% dioctylsulfosuccinate in sirupus simplex DAB 10. The preparation was similar to formulation B.
F. Spironolactone suspension with surfactant consisted of 10% nonmicronized spironolactone, 0.5% dioctylsulfosuccinate in sirupus simplex DAB 10. The preparation was similar to formulation B.
G. Spironolactone nanosuspension SLN-1 consisted of 0.3% spironolactone and 29.7% Dynasan 114 as lipidic phase, 2.5% Tween 80 and 67.5% demineralized water as hydrophilic phase. An emulsion

was prepared at 80°C using a Polytron, which was passed through the high pressure homogenizer for 20 minutes at a pressure of 500 bar and 80°C. The mean particle size of the nanosuspension determined by PCS was 253 nm. The distribution was: 10% <0.085 μm, 50% <0.262 μm, and 90% <0.399 μm.

H. Spironolactone nanosuspension SLN-2 consisted of 0.5% spironolactone, 9.5% Vitamin E TPGS, 10% d-alpha tocopheryl acetate (Vit E6-100), and 80% demineralized water. The manufacturing process was similar to the process described for SLN-1. Particle size analysis determined by LD yielded by volume: 10% <0.049 μm, 50% <0.065 μm, and 90% <0.085 μm, the average particle size was 41 nm (PCS).

All concentrations given are w/w. The yield of each process in general was better than 80%. All formulations were confirmed to be physically stable for at least 2 months, a time frame that allowed completion of all pharmacokinetic studies. Content uniformity of all formulations passed the requirements of USP 27/NF 22 (2004).

Pharmacokinetic Study

The study was reviewed and approved by the animal committee of Martin-Luther University and by the Regierungspräsidium Halle. Blood samples (150 μL) were drawn from the tail vein via an implanted catheter (modified Harvard-Swivel model). Implantation was under ketamine/rompun (50 mg/kg and 10 mg/kg, respectively) anesthesia 24 h prior to commencement of the kinetic study. The pharmacokinetic study was carried out in male Wistar rats (Charles River, Sulzfeld, Germany). They were housed in cages for a minimum of 3 days prior to the begin of the study. The body weight for animals to be included in the study was 200–250 grams. Food was Altromin® standard diet and was withdrawn from the animals 10 to 12 hours prior to the beginning of the study, and until 2 hours post dosing. The animals, however, had free access to water. All animals were randomly assigned to a study group. Each study group consisted of three to six animals and received one of the formulations as described above. Table 1 presents an overview of the studies performed and formulations tested.

Following i.v. administration, blood was sampled at the following time points: 10′, 20′, 30′, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h (10 samples). Serum was obtained from whole blood by centrifugation at 10′000 g after clotting for 1 h at 4°C. Thereafter, the serum samples were frozen until analysis.

For the peroral administration of the nanosuspension type DissoCubes®, the nanosuspension was diluted 1:10 with saline prior to administration. The administration volume was based on the dose to be administered and the individual body weight of each animal. The same procedure was applied in the case of the suspension and microsuspension formulations. In the case of the solid lipid nanosphere suspensions, they were dosed without prior dilution with sodium chloride solution. Blood was sampled at the following time points: 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 22 h (10 samples). Following the blood sampling, serum was obtained from whole blood by centrifugation at 10′000 g after clotting for 1 h at 4°C. Thereafter, the serum samples were kept frozen at −20°C until analysis.

Analysis

Blood serum was assayed for spironolactone, canrenone, and 7-α-thiomethylspirolactone modified on the basis of a published method using HPLC

TABLE 1 Overview of Spironolactone Formulations and Doses Tested

Formulations and doses[a] tested							
Solution i.v.	Disso-Cubes®	Microsuspension	Microsuspension +surfactant	Suspension	Suspension +surfactant	SLN 1	SLN 2
5	20	–	–	20	–	–	–
–	50	50	50	50	50	50	50
–	100	–	–	100	–	–	–

[a]Doses are given in mg/kg body weight.

with UV-VIS detection (Kaukonen et al., 1998). HPLC-Pump, automatic sampler, and UV-detector were from Jasco, Groß-Umstadt, Germany. The column was a Spherisorb ODS-2 (5 µm , 250 × 4 mm), Bischoff, D-Leonberg, precolumn a Spherisorb (4 × 4 mm), Bischoff, D-Leonberg. The flow rate was 0.9 mL/min. The mobile phase consisted of 70% methanol in water. UV detection was at 238 nm for spironolactone, 7α-thiomethylspirolactone and 280 nm for canrenone. Unchanged spironolactone was only detected in very few samples, particularly after i.v. dosage. Therefore, the pharmacokinetic analysis was based on the measured concentrations of canrenone and 7-α-thiomethylspirolactone. The amount of serum analyzed was 100 mg per sample, which were weighed into Eppendorf caps. Thereafter, 100 µL acetonitrile und 45 µL of internal standard (progesterone 10 µg/mL) were added, the sample was vortexed for 15 s and centrifuged for 5 min at 5000 rpm. The clear supernatant was pipetted into an autosampler vial and 100 µL were injected into the HPLC. The chromatograms were analyzed using Borwin Software and Microsoft Excel. Calibration curves of spironolactone, canrenone, and 7-α-thiomethylspirolactone were run over a concentration range of 50 ng/g up to 5000 ng/g sample with reference substance prior to each run of test samples. Weighted linear regression analysis was performed using $1/x^2$ as weighting factor. For spironolactone, the precision of the analytical method was 4.6% (CV) at 50 ng/mL and 2.1% (CV) at 5000 ng/mL. For 7-α-thiomethylspirolactone the precision was 7.1% at 50 ng/mL and 1.5% at 5000 ng/mL. For canrenone the precision was 4.2% at 50 ng/mL and 2.5% at 5000 ng/mL. The limit of detection was estimated at 20 ng/mL, the lower limit of quantification was defined as 50 ng/mL. The calibration curves were linear for the parent compound and the metabolites.

Quantitative analysis of spironolactone from samples of dissolution fluid was carried out using an HPLC pump, automatic sampler, and UV-detector from Series 1050, Hewlett Packard, data processing was by HPLC Chemstation Rev. A. 02.05 (Hewlett Packard). UV absorbance was measured at 238 nm. The injection volume was 10 µL. The stationary phase was Nucleosil 100 C-18 material, 5 µm particle size, 25 × 4 mm (Phenomenex). The mobile phase was 50 mM sodium phosphate buffer pH 3.5, 40% and acetonitrile, 60%. The flow rate was 0.8 mL/min. Calibration curves were constructed by dissolving spironolactone in 0.1 M HCl. The concentration range was 0.16–8.0 µg/mL.

Dissolution

Dissolution of active pharmaceutical ingredient from suspension formulations was performed using the USP Apparatus 2 setup (paddle method). An aliquot of the suspension was pipetted into the dissolution vessel containing 900 mL 0.1 M HCl at 37°C. The rotation speed of the paddle was set to 75 rpm. The sample volume was 500 µL at each time point. Sample preparation was as follows: 500 µL of dissolution sample fluid containing solid particles were pipetted into a Microcon YM-30 centrifugal filter device (Millipore, Eschborn, Germany). The device is used for simple and efficient concentration of macromolecular solutions by centrifugation at 14′000 rcf at 25°C in an Eppendorf centrifuge Type 5804R using a

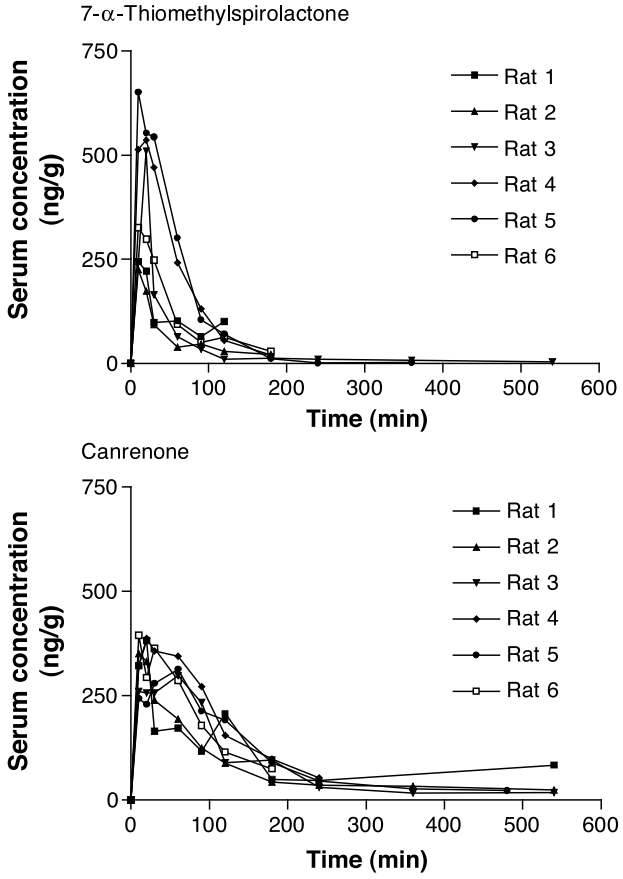

FIGURE 1 **Individual Blood Serum Concentration-Time Profiles of 7-α-Thiomethylspirolactone (Upper) and Canrenone (Lower) Following i.v. Dosing of Spironolactone at 5 mg/kg. Average t_{max} were 13.3±5.8 min and 30.0±26.5 min for 7-α-Thiomethylspirolactone and Canrenone, Respectively.**

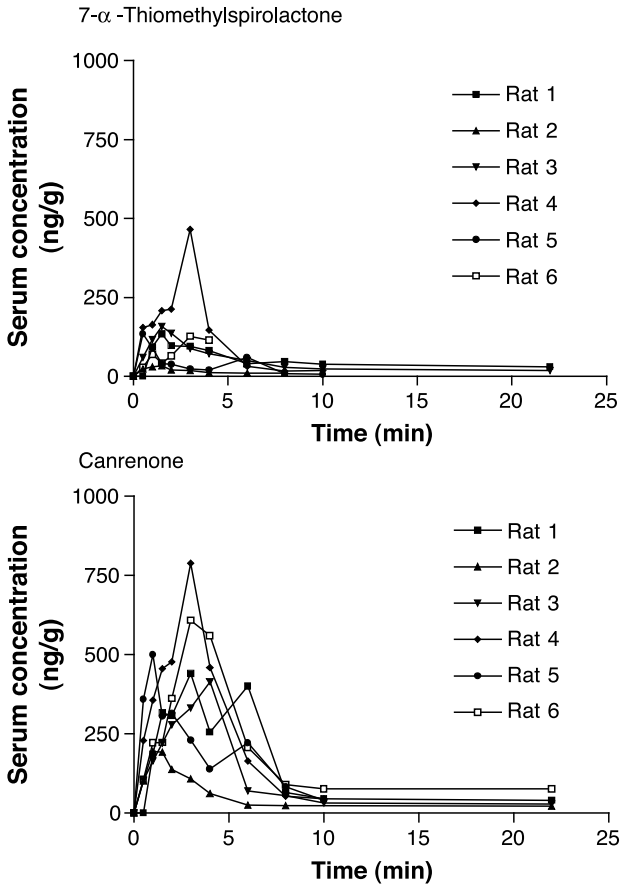

FIGURE 2 **Individual Blood Serum Concentration-Time Profiles of 7-α-Thiomethylspirolactone (Upper) and Canrenone (Lower) Following p.o. Dosing of Spironolactone Suspension at 50 mg/kg.**

fixed angle rotor type FA 45-30-11. The centrifugation time was 3 minutes. It could be shown that this particular method of sample cleanup was successful in separating the nanoparticles from the dissolved spironolactone in the dissolution fluid. The binding of spironolactone to the filter material was negligible. Samples were taken from 1 minute up to 60 minutes and centrifuged immediately afterwards. Except for the SLN formulations, which were not retained by the filter due to their low particle size, all other suspensions were successfully characterized by this method.

Data Analysis

Standard pharmacokinetic parameters (±SD) were calculated according to noncompartmental procedures using WinNonlin Professional (Pharsight Corporation, Mountain View, USA) and TOPFIT 2.0 (Gustav Fischer, Stuttgart). Statistical comparisons were performed using Microsoft Excel 2000 Analysis Tool Pack and S-Plus 2000 (MathSoft Inc. Seattle, WA). Statistical testing of differences between two mean values was performed using the two-sided t-test for samples with unequal variance, with α set to 0.05. One-way analysis of variance (ANOVA) was performed for testing differences between means for more than two groups for statistical significance with α set to 0.05.

RESULTS

Very low concentrations of parent spironolactone were found only in a few serum samples following i.v. dosing, whereas no parent compound was determined following p.o. administration. Instead, serum concentrations of phase-I metabolites were employed for establishing a biopharmaceutical comparison of the various spironolactone formulations. Individual serum concentration vs. time curves of 7-α-thiomethylspirolactone and canrenone following intravenous dosing of spironolactone at 5 mg/kg are shown in Fig. 1. For

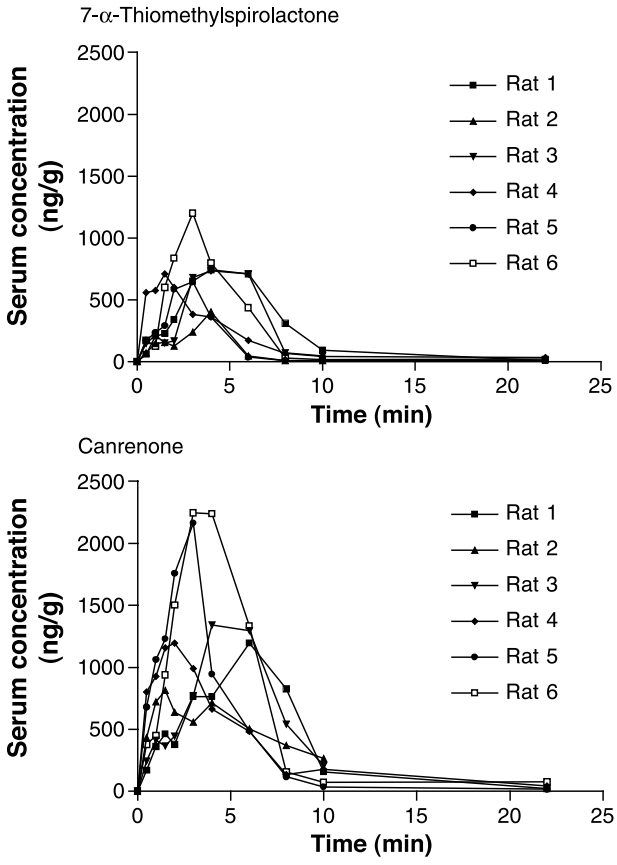

FIGURE 3 **Individual Blood Serum Concentration-time Profiles of 7-α-Thiomethylspirolactone (Upper) and Canrenone (Lower) Following p.o. Dosing of Nanosuspension Type DissoCubes® at 50 mg/kg.**

TABLE 2 Ratios of $AUC_{0-tlast}$, $AUC_{0-\infty}$ and C_{max} Determined Following Administration of Spironolactone Formulations Over the Reference Spironolactone Suspension

Formulation	Compound analyzed	R $AUC_{0-tlast}$	R $AUC_{0-\infty}$	R C_{max}
Nanosusp. DissoCubes®	7-α-Tms	5.11±4.11[b]	3.58±2.58[b]	4.46±4.43[b]
	Canrenone	3.31±1.61[b]	3.28±1.39[b]	3.04±1.70[b]
Microsuspension+surfactant	7-α-Tms	6.74±5.43[b]	4.48±3.24[b]	8.05±8.54[b]
	Canrenone	3,84±2.12[b]	3.60±1.66[b]	3.50±1.77[b]
Microsuspension	7-α-Tms	1.61±2.59	1.16±1.67	1.71±2.95
	Canrenone	1.17±1.26	1.15±1.07	1.17±1.62
Suspension+surfactant	7-α-Tms	9.55±8.80[b]	6.94±5.79[b]	6.42±7.52[a]
	Canrenone	4.31±2.64[b]	4.99±2.30[b]	3.11±1.66[b]
SLN 0.5%	7-α-Tms	15.1±14.72[b]	10.1±9.06[b]	17.61±17.66[b]
	Canrenone	5.69±3.51[b]	5.20±2.89[b]	5.49±2.58[b]
SLN 0.3%	7-α-Tms	1.64±1.26	1.21±0.81	0.56±0.62
	Canrenone	0.96±0.45	1.35±0.57	0.60±0.30

For each formulation, a dose of 50 mg/kg was administered. Given are the ratios of the mean parameter values. The standard deviation was calculated according to the Gaussian error propagation law.

[a] $p<0.05$.

[b] $p<0.01$.

comparison, individual concentration-time profiles following p.o. administration of spironolactone suspension and nanosuspension type DissoCubes® are shown in Figs. 2 and 3, respectively.

Ratios of $AUC_{0-tlast}$, $AUC_{0-\infty}$, and C_{max} of peroral vs. intravenous administration allow an estimate of the systemic availability of the active principle although, when estimates are based solely on metabolite ratios,

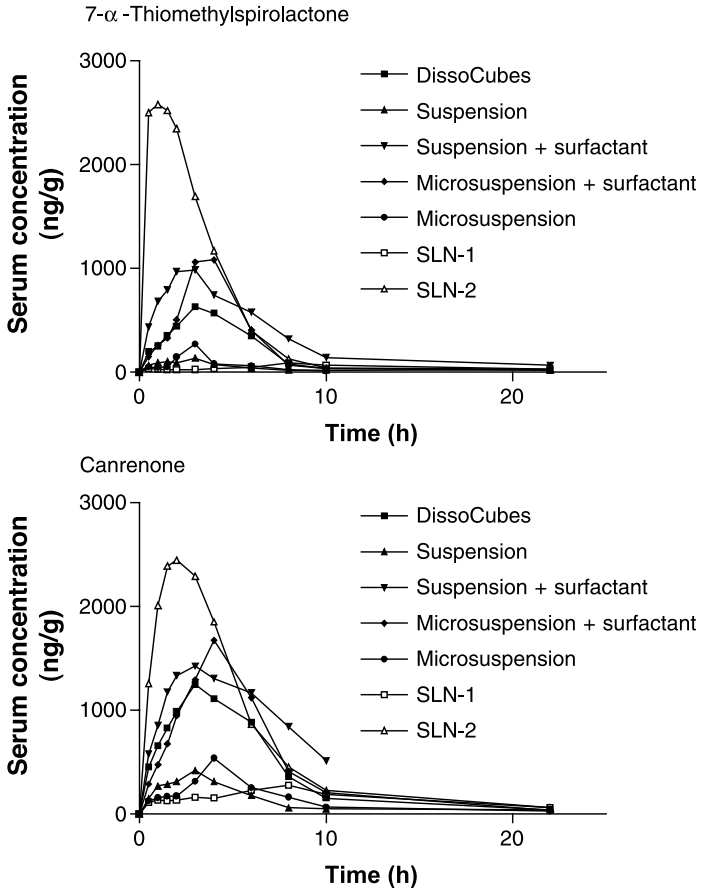

FIGURE 4 Mean Blood Serum Concentration Time Profiles of 7-α-Thiomethylspirolactone (Upper) and Canrenone (Lower) Following Peroral Dosing of Spironolactone at 50 mg/kg in Different Formulations (n=3–6).

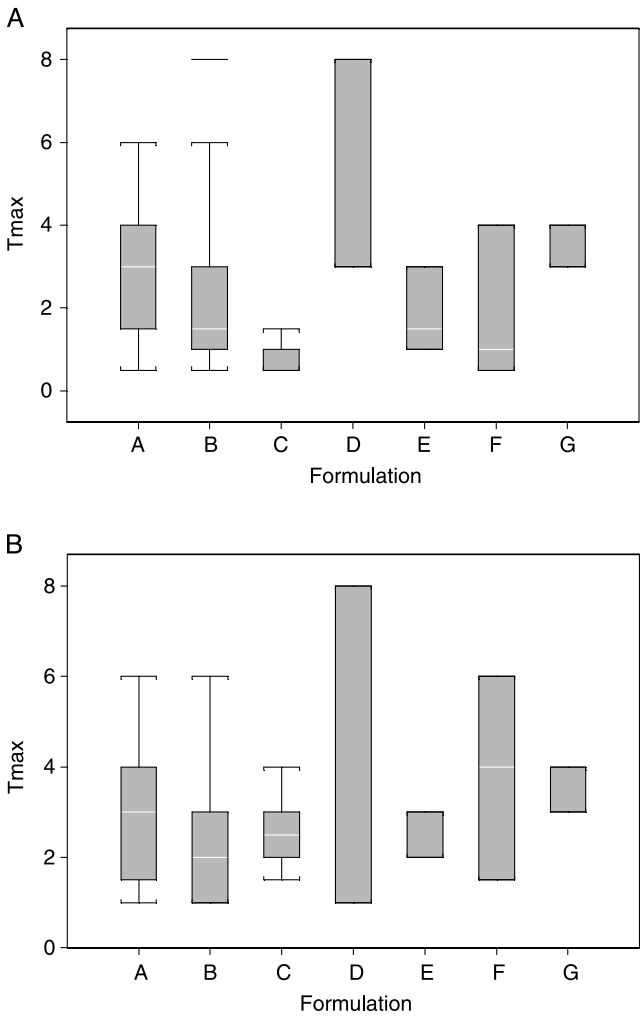

FIGURE 5 **A. Box-and-Whisker Plot of t_{max} Values (in hours) of 7-α-Thiomethylspirolactone Concentration Time Curves Following p.o. Administration of Spironolactone Formulations: A: Nanosuspension DissoCubes®, B: Suspension, C: SLN-2, D: SLN-1, E: Suspension + Surfactant, F: Microsuspension ithout Surfactant, G: Microsuspension with Surfactant. B. Box-and-Whisker Plot of t_{max} Values (in h) of Canrenone Concentration Time Curves Following p.o. Administration of Spironolactone Formulations: A: Nanosuspension DissoCubes®, B: Suspension, C: SLN-2, D: SLN-1, E: Suspension + Surfactant, F: Microsuspension, G: Microsuspension + Surfactant.**

inaccuracies due to the hepatic first-pass effect are inevitable and bioavailability may be overestimated. For the spironolactone suspension the extent of systemic availability ranged from 18% to 33% based on the ratios of $AUC_{0-tlast}$ and $AUC_{0-\infty}$ for canrenone, respectively, and from 32% to 33% based on the ratios of $AUC_{0-tlast}$ and $AUC_{0-\infty}$ for 7-α-thiomethylspirolactone. With some formulations, e.g., microsuspension + surfactant, suspension + surfactant, SLN-2, the ratio of AUC values exceeded unity for reasons stated above (data not shown). Ratios of $AUC_{0-tlast}$, $AUC_{0-\infty}$, and C_{max} of p.o. test formulations over the suspension as reference formulation are given in Table 2. The best improvement in bioavailability was observed for the solid lipid nanosuspension SLN-2. Increase in AUC of canrenone was 5.7-fold and 15.1-fold for 7-α-thiomethylspirolactone. The improvement of bioavailability was similar based on the C_{max} metric. The DissoCube® nanosuspension

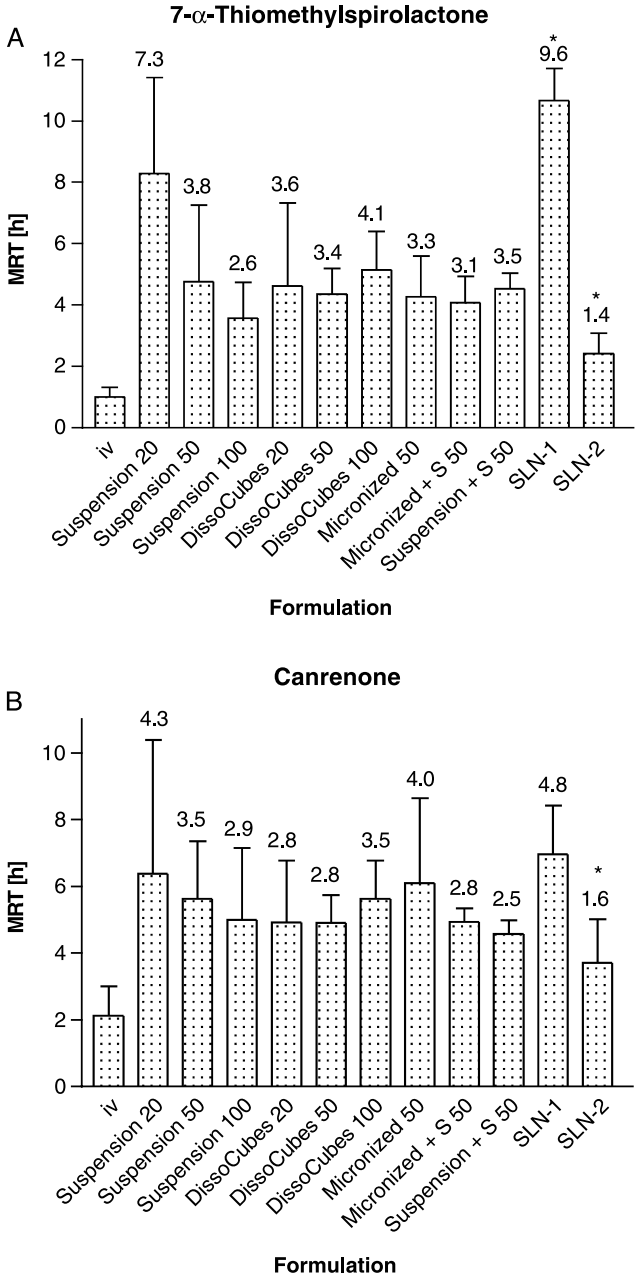

FIGURE 6 **A. Mean Residence Times of 7-α-Spirolactone Following Intravenous and Oral Administration of Spironolactone in Various Formulations. The Numbers Above Each Bar Indicate the Mean Absorption Times (in hours) of the Drug from the Formulations. B. Mean Residence Times of Canrenone Following Intravenous and Oral Administration of Spironolactone in Various Formulations. The Numbers Above Each Bar Indicate the Mean Absorption Times (in hours) of the Drug from the Formulations.**

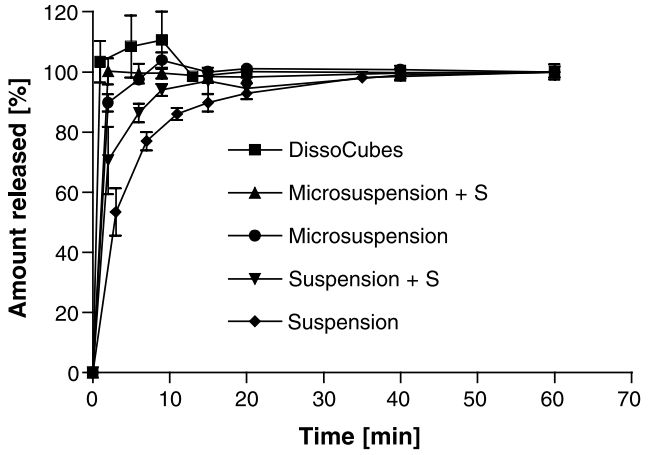

FIGURE 7 **Cumulative Dissolution of Spironolactone from Selected Nanosuspensions, Microsuspensions and Suspensions (Paddle-Method, 75 rpm, 37°C, 900 ml 0.1 M HCl).**

yielded also highly significant improvements in bioavailability ranging from 3.3- to 5.1-fold in AUC and 3.0- to 4.5-fold in terms of C_{max}. Significance testing revealed that in all cases, significant differences between the test and reference formulations were observed for both metabolites, i.e., for 7-α-thiomethylspirolactone and for canrenone. Very little improvement in bioavailability was observed for the suspension containing micronized spironolactone. Similarly, the solid lipid nanosuspension SLN-1 did not show a significant improvement over the suspension containing the coarse spironolactone material. Interestingly, the addition of surfactant to coarse drug material as well as to micronized spironolactone resulted in a significant increase in the bioavailability parameters AUC and C_{max}. This underlines the importance of the presence of surfactant in formulations of spironolactone, whereas the factor particle size seems to be of minor relevance. The average concentration vs. time profiles of the two spironolactone metabolites 7-α-thiomethylspirolactone and canrenone following administration of all investigated formulations are depicted in Fig. 4.

The analysis of t_{max} parameters also revealed interesting insights. Figures 5A and B show the statistical distribution of t_{max} values of 7-α-thiomethylspirolactone and canrenone for the seven peroral formulations tested. ANOVA testing on the mean t_{max} values for 7-α-thiomethylspirolactone indicated statistically significant differences between the group means ($P=0.0024$). Multiple comparisons between group means showed that the t_{max} of the SLN-2 formulation is significantly shorter than that of the DissoCube® nanosuspension and the suspension. None of the other multiple comparison tests showed statistically significant differences, although the comparison between t_{max} values of SLN-2 vs. SLN-1 was at the borderline of statistical significance. In the case of canrenone, analysis of variance indicated no significant differences

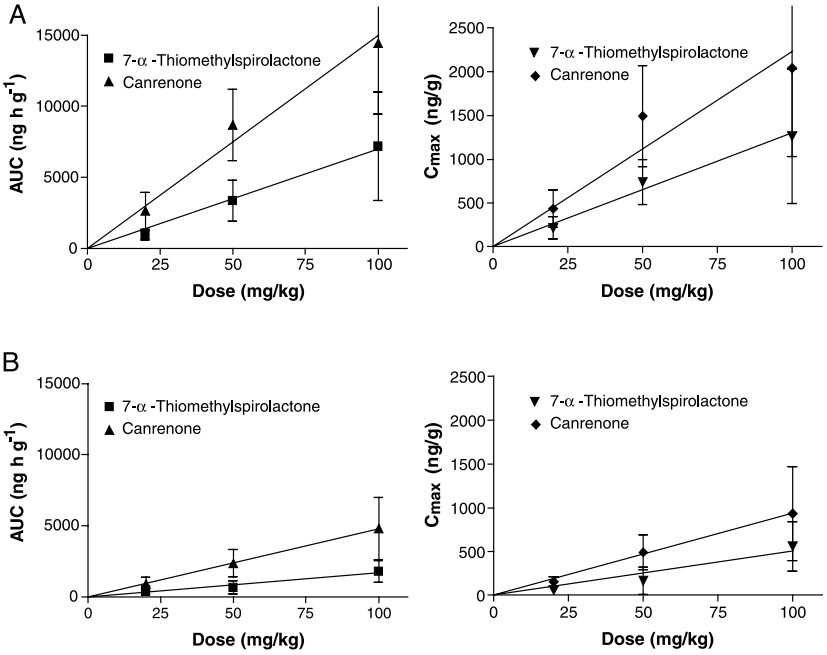

FIGURE 8 **A. Dose Dependence of $AUC_{0-tlast}$ and C_{max} Following Peroral Dosing of the Nanosuspension DissoCubes® in Fasted Rats. Means ± SD, n = 6. B. Dose Dependence of $AUC_{0-tlast}$ and C_{max} Following Peroral Dosing of Spironolactone Suspension in Fasted Rats. Means ± SD, n = 6.**

between group means of all tested formulations (P=0.053). Analysis of the absorption velocity by mean time analysis yielded a similar result. In Fig. 6 the mean residence times of 7-α-thiomethylspirolactone and canrenone are shown. Only in the case of the SLN-2, significantly shorter mean residence times of the metabolites were detected following peroral administration, indicating an increased absorption velocity of active compound from this formulation. This is an interesting phenomenon, since the DissoCube® nanosuspension, although also improving the exposure of the body to the drug, did not show an increased absorption velocity of active. Rather, the absorption velocity was similar to the other formulations containing dioctylsulfosuccinate as surfactant.

Results from the dissolution study demonstrate that all the formulations investigated dissolve rapidly (>80% in less than 15 minutes). Typical cumulative dissolution profiles are shown in Fig. 7. The most rapid dissolution of spironolactone was provided by the DissoCube® nanosuspension, whereas the suspension without surfactant added was the slowest dissolving formulation tested. It becomes apparent that particle size and the presence of surfactant have an impact on the dissolution rate. The suspensions with micro- or nanosized spironolactone material showed significantly faster dissolution rates than the suspensions with coarse drug material. Similarly, the presence of surfactant caused a significant increase in the rate of dissolution of spironolactone, as demonstrated by the comparison of spironolactone suspension vs. spironolactone containing surfactant.

The dose dependence of the bioavailability parameters was investigated at 20, 50, and 100 mg/kg for the nanosuspension DissoCubes® and for the suspension without surfactant. A linear increase in the $AUC_{0-tlast}$ as well as C_{max} with dose was observed for both 7-α-thiomethylspirolactone and canrenone, indicating nonsaturable input processes for both formulations.

The slopes of the lines were larger for the nanosuspension indicating the greater potential of this dosage form to deliver higher doses of low soluble compound systemically. The data are shown in Fig. 8.

DISCUSSION

Experience with the in vivo performance of nanosized delivery systems for low soluble drugs is still limited today. The present study was designed to investigate the influence of surfactant and particle size on the pharmacokinetics and in particular the absorption of spironolactone. Since spironolactone is undergoing efficient first-pass extraction, two of its major metabolites, 7-α-thiomethylspirolactone and canrenone, were used as surrogates for the biopharmaceutical evaluation. In addition, spironolactone has been administered intravenously as a solution in a mixture of cosolvents and buffer, in order to obtain the formation and disposition parameters of both metabolites.

Pharmacokinetic analysis revealed a systemic availability of spironolactone of 18–24% (in terms of 7-α-thiomethylspirolactone) and 32–33% (in terms of canrenone). These estimates were based on an i.v. dose of 5 mg/kg and a peroral dose of 50 mg/kg (suspension of coarse drug material). More rapidly dissolving formulations yielded systemic availabilities exceeding 100%. This was most probably due to the fact that metabolite concentrations following peroral dosing exceed those following intravenous administration, even when equal doses are administered. In the literature, the systemic availability of radiolabeled spironolactone in terms of parent compound and metabolites at 5 mg/kg i.v. and p.o. doses was estimated to be 82% in the rat (Karim et al., 1976).

Relative systemic availabilities of spironolactone in terms of its metabolites were formulation-dependent based on the results of the present study. Bioavailability improvement was best for the solid lipid nanosuspension SLN-2 when compared with the suspension containing coarse drug material. Interestingly, the improvement in bioavailability was in most cases higher for 7-α-thiomethylspirolactone parameter ratios than for ratios calculated for canrenone. The reason for this observation is yet unclear. It should be kept in mind, though, that the biotransformation pathways of spironolactone are complex and some metabolites still remain to be identified. It is also known that both 7-α-thiomethylspirolactone and canrenone are only intermediates in the biotransformation cascade of spironolactone, i.e., they are metabolized further to other metabolites (Boreham et al., 1981; Dollery, 1999). The serum concentration of the metabolite is dependent on both its formation and elimination kinetics. Little is known, however, about the potential saturability of these metabolism pathways, and consequently it may

well be assumed that they may not be the same for both metabolites.

Seven peroral formulations of spironolactone have been compared in the present study. The results in terms of bioavailability improvement may be grouped into three classes. The largest improvement was seen with the SLN-2 formulation, followed by a group of three formulations containing dioctylsulfosuccinate as surfactant, i.e., nanosuspension DissoCubes®, microsuspension + surfactant and suspension + surfactant. The DissoCube® nanosuspension also contains considerable amounts of surfactant as stabilizer in order to inhibit crystal growth. The third class, which includes all other formulations, showed very little or no improvement in bioavailability. Rather, the plasma concentration-time profiles from this group of formulations were—to a high degree—erroneous and variable.

Analysis of t_{max} data showed that only in the case of SLN-2, a higher absorption velocity was reflected in significantly shorter t_{max} values. Similarly, mean time analysis indicates a significantly shorter mean absorption time of the active species for formulation SLN-2.

In addition to SLN-2, several other formulations also demonstrated improvements in bioavailability (exposure) over the suspension formulation; however, their t_{max} values as well as their mean absorption times were not significantly different from the suspension formulations as reference values.

Several hypotheses may be generated to explain these interesting experimental findings. The results from the dissolution studies demonstrate a rank order in vitro/in vivo correlation between the in vitro drug release and the in vivo pharmacokinetic parameters AUC and C_{max} of spironolactone metabolites. This indicates that the differences in bioavailability can in part be attributed to a more rapid dissolution of active pharmaceutical ingredient from the formulation. Generally, those formulations that gave faster in vitro release rates tend to yield a higher area under the curve and C_{max} values for 7-α-thiomethylspirolactone and canrenone. Although the differences in release rates of spironolactone between the formulations were not major, it must be taken into account that in vitro dissolution conditions were set to reflect sink conditions. In vivo, however, in particular at high doses, these sink conditions may not prevail in the intestine and differences between the formulations—in terms of dissolution rates as well as drug solubilities—may be more expressed than what is reflected by the differences in the current in vitro dissolution data. An increase in drug solubility in the intestine—due to the presence of surfactants—may equally well serve to explain the observed phenomena. Increases in solubility as well as enhanced membrane permeability across Caco-2 cell monolayers has been demonstrated for nanosuspensions type DissoCubes® with spironolactone as well as with other active pharmaceutical ingredients such as nifedipine and budesonide previously (Ohlig et al., 2001). Thus, the in vivo studies presented here demonstrate nicely the in vivo relevance of the previous in vitro findings.

Nevertheless, additional direct effects of the excipients included in the formulations on the intestinal permeability of spironolactone can also not be excluded at present. This holds true e.g., for d-α-tocopheryl polyethylene glycol 1000 succinate (Vitamin E TPGS) in formulation SLN-2, a surfactant that has been described to improve the intestinal permeability and solubility of the HIV protease inhibitor amprenavir (Yu et al., 1999). More detailed investigations on the mechanism of intestinal permeation of spironolactone and the influence of Vitamin E TPGS will possibly shed some light on the observed effects. Furthermore, nanosized drug material may directly interact with the intestinal epithelium leading to 1) decreased drug transit times through the GI tract with the possibility of a prolongation of drug dissolution and/or 2) direct uptake of the nanoparticles into the GI epithelium. Both aspects, however, are still at debate today and direct interaction of nanoparticles with the intestinal epithelium may also be the result of a particular design of the in vitro study rather than be an experimentally well defined phenomenon (Pietzonka et al., 2002a, 2002b).

Another aim of the present study was to challenge the dose-linearity relationship of the pharmacokinetics of spironolactone metabolites in the dose range from 20 to 100 mg/kg. As a working hypothesis it was speculated that the spironolactone nanosuspension would show an improved dose-linearity relationship over the suspension of coarse spironolactone material, due to faster dissolution rate and higher solubility of the drug. However, analysis of data suggested that, although the nanosuspension showed consistently higher bioavailabilities in terms of AUC and C_{max} at all doses investigated, the PK parameters behaved

linearly with increasing doses for both formulations investigated.

In summary, several of the newly developed nanosuspensions showed increased bioavailability over the reference formulation containing coarse spironolactone. This finding is not unusal, since improvements in bioavailability with nanosized drug delivery systems have been reported by other authors as well. For example, a roughly four-fold improvement in C_{max} was found for a poorly soluble compound in the dog when the mean particle size was reduced from 5.0 μm to 0.1 μm (Merisko-Liversidge et al., 2003). Furthermore, De Jaeghere et al. (2001) reported an improvement by a factor of 4.2-fold in terms of AUC for a highly lipophilic drug formulated in poly(methacrylic acid-co-ethylacrylate) nanoparticles. Another observation in conjunction with the newly developed formulations, the DissoCubes® nanosuspension and the solid lipid nanosuspension SLN-2 was their superior handling as compared to the formulations with coarse drug material. This was due to the more homogeneous distribution of nanoparticles within the suspension, such that a more accurate dosing was possible with less vigorous shaking. Nanosuspensions consequently represent a promising alternative to current delivery systems aiming to improve the biopharmaceutic performance of drugs with low water solubility.

CONCLUSIONS

This study has confirmed that some nanosized drug delivery systems are suitable for improving the bioavailability of poorly water soluble drugs. In addition to particle size, the presence of surfactants in the formulations had a major impact on bioavailability parameters. These findings confirm general strategies for the formulation of low soluble compounds in oral dosage forms and point the direction towards the pharmaceutical development for class II and IV compounds of the Biopharmaceutics Classification System.

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