Improving Relative Bioavailability of Dicumarol by Reducing Particle Size and Adding the Adhesive Poly(Fumaric–Co-Sebacic) Anhydride

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Purpose. This study was carried out to show the effect of particle size reduction and bioadhesion on the dissolution and relative bioavailability of dicumarol.

Methods. Formulations were produced by a variety of methods including a novel technique to reduce particle size as well as phase inversion with poly(fumaric–co-sebacic)anhydride p(FA:SA) to create nanospheres. Drug was administered to groups of pigs and rats via oral gavage of a suspension, and dicumarol concentration in the blood was measured using a double extraction technique.

Results. *In vitro* results showed improved dissolution in both the micronized formulation and the encapsulated p(FA:SA) nanospheres. *In vivo,* relative bioavailability of a spray-dried formulation was increased by 17% in the rat and 72% in the pig by further reduction in particle size. The bioadhesive p(FA:SA) formulation also improved relative bioavailability over the spray-dried drug, increasing it by 55% in the rat and 96% in the pig. Additionally, the p(FA:SA) formulation prolonged T_{max} and decreased C_{max} in both species.

Conclusion. This work demonstrates the importance of particle size and bioadhesion to improve oral bioavailability of ducumarol.

KEY WORDS: reducing particle size; solid solution; poly(fumaric–co-sebacic) anhydride; bioadhesion; poorly water-soluble drug.

INTRODUCTION

New breakthroughs in drug discovery and high-throughput screening have led to a wealth of drugs that are poorly water soluble and suffer from poor oral bioavailability (BA). The oral BA of poorly soluble drugs is affected by a variety of factors, most importantly permeability and solubility. The ability to permeate and penetrate into the systemic circulation can be considered an inherent attribute of the drug, but some groups have attempted the addition of penetration enhancers to drug formulations to improve permeability. The results have not been encouraging (1), and thus, most work in formulation development has focused on manipulating dissolution rate.

There are numerous physical techniques that are currently used to improve the dissolution of hydrophobic drugs, and most are based on the principle that with greater surface area of drug exposed, a more rapid dissolution can occur. This comes directly from the Noyes-Whitney equation (2) for dissolution of solids, which states that the rate of dissolution is directly proportional to surface area. Current methods used to improve solubility include carrier complexation (3,4), formation of a molecular dispersion via a solid solution (5), using a drug in its amorphous state (6), reduction in particle size (1,7,8), and others. Carrier complexation is primarily used to combine certain molecules, including a variety of water-soluble polymers, to the drug in an attempt to increase solubility either by promoting dissolution of the carrier or by creating a dispersion of the drug. Such dispersions can potentially separate individual molecules of the drug, greatly increasing the surface area and rate of dissolution. If a true molecular dispersion is obtained, or a solid solution, then the overall crystalline character of the drug will be diminished, creating a more amorphous system with much faster dissolution. A similar effect can also be achieved by precipitating a drug followed by rapid quenching (8), which occurs in the widely used process of spray drying.

Recent reports in the literature have demonstrated the benefits in improved bioavailability from formulating drugs into one of these forms. Lee *et al.* have shown a 1.7-fold increase in the AUC of cyclosporin A dispersed in SLS-dextrin microspheres (9).

Halofantrine was formulated into various solid dispersions by Khoo *et al.,* and the work showed a five- to sevenfold increase in absolute bioavailability (10). And in our group, Chickering *et al.* (11) have shown enhancement of the bioavailability of dicumarol using bioadhesive polymers encapsulating spray-dried dicumrol formulations. Many other examples exist in the literature, and the apparent popularity of formulating drugs in this manner is obviously well deserved. One interesting example of how to formulate dicumarol with defatted milk into a solid solution is presented by Macheras *et al.* (12).

In this study, we have prepared and evaluated, both *in vitro* and *in vivo*, several formulations with reduced particle size. Relative bioavailability was determined in both rats and pigs as compared to spray-dried dicumarol. The effect of particle size and bioadhesion was evaluated, and their effect on T_{max} and C_{max} is discussed.

MATERIALS AND METHODS

Dicumarol and Reagent Source

Dicumarol was purchased from Sigma-Aldrich and was stored at room temperature. Coulter particle analysis showed that the mean particle diameter was 18.5μm based on volume statistics. All reagents and solvents used throughout the study were purchased from either Fisher or Mallinckrodt and were of the highest grade available.

Poly(Fumaric–Co-Sebacic) Anhydride Synthesis

The polymer used throughout this study is the polyanhydride poly(fumaric-co-sebacic) anhydride [p(FA:SA)] and was synthesized in our laboratory using melt polycondensation according to methods previously described (13–15). A Bruker DPX300 NMR was used for one-dimensional proton NMR analysis. The polymer in deuterated chloroform was analyzed using peak ratios of the olefinic protons of the fumaric acid monomer (δ = 6.91 and 6.97) and the internal aliphatic protons of the sebacic acid monomer (δ = 1.32). The

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normalized molar ratio was determined to be FA:SA 17:83. For analysis of molecular weight, a 5% solution of p(FA:SA) in chloroform was analyzed on a Perkin Elmer LC pump model 250 gel permeation chromatography system composed of an isocratic LC pump, model 250; an LC column oven, model 101; an LC-30 RI detector; and a 900 series interface computer. Samples were eluted through a PL gel 5-μm mixed column and a 5-μm/50-Å column connected in series at a flow rate of 1.0 mL/min and a temperature of 40°C. The system was calibrated with a series of monodisperse polystyrene standards (MW: 600–200,000) in chloroform, and the molecular weight of p(FA:SA) was found to be 12 kDa. The polymer was stored under a nitrogen purge at –20°C until use.

Scanning Electron Microscopy

All samples were sputter coated with an Au-Pd target for 3.5 min and spread over a carbon-backed adhesive disk on top of the SEM stub. The Hitachi 2700 was used to visualize the samples at an accelerating voltage of 8 kV.

Differential Scanning Calorimetry

A Pyris 1 DSC with an Intercooler 2P cooling system was used to thermally characterize the formulations. Following a baseline run at a heating and cooling rate of 10°C/min from 0 to 320°C, 5-mg samples were hermetically sealed in aluminum pans and run under a nitrogen purge using the same parameters.

Formulating Dicumarol

Particulates of dicumarol were produced using two techniques yielding different size distributions. One method was used to produce submicron particles, and the other produced particles with a median diameter of approximately 3 μm.

Spray drying was used to create the 3-μm formulation. Twenty grams of dicumarol was dissolved in 8 L methylene chloride to make a 0.25% (w/v) solution. This solution was spray dried in a Lab Plant SD-04 Laboratory spray drier using a pressure pot at a pressure of 68 psi, an atomizer pressure of 65 psi, and a solvent flow rate of 30 mL/min. The drying temperatures of the inlet and outlet were 45°C and 24°C, respectively. The spray-dried microparticles (SD) were collected from of the walls of the device, lyophilized, and stored at –20°C until further use.

The submicron particulates were produced using a novel technique: 330 mg dicumarol was dissolved in 30 mL dimethylsulfoxide with a micro magnetic stir-bar rotating at 900 rpm. The temperature of the solution was raised until dissolution occurred, which was typically around 100°C. The entire volume of this solution was dispersed in 500 mL isopropyl alcohol, creating a two-phase system. After vigorous stirring, 600 mL distilled water was added in a stream, resulting in a colloidal dispersion of a milky precipitate. With a cylindrical pressure filtration apparatus, the nanoparticles were collected on 100-nm filter paper composed of mixed cellulose esters. The powder was then frozen and lyophilized for 48 h.

To study the effect of encapsulation on the micron-sized drug, the polymer p(FA:SA) 17:83 was used to produce nanospheres by the phase-inversion method: 100 mg of submicron dicumarol particulates was probe sonicated for 3 min at amplitude 35% in 20 mL methylene chloride, causing complete dissolution of the drug. Then 100 mg p(FA:SA) 17:83 was dissolved in this solution by sonication for an additional 30 s. The resultant solution was dispersed into 1.0 L petroleum ether, and the precipitate was collected using a 100-nm filter composed of mixed cellulose esters. The microsphere formulation was then frozen and lyophilized for 24 h. The dicumarol loading was determined in this formulation by a simple extraction protocol. Microspheres were incubated overnight in 2.5 N NaOH at 37°C. On dissolution, an aliquot of approximately 15 μg based on theoretical loading was added to 2.5 N NaOH to make a total volume of 800 μL. This mixture was agitated for 2 min and centrifuged for 2 min at 11,269 *g*. The supernatant was removed and analyzed on the Shimadzu UV-2501 spectrophotometer and compared to a linear standard curve of dicumarol in NaOH.

Particle Sizing

All microspheres and particulate formulations were sized using laser diffractometry via the Coulter Particle Size Analyzer LS 230. A 250 μg/mL suspension of microspheres in 1% pluronic F127 [poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide)]/1% hydroxypropylmethylcellulose (HPMC) was introduced into the small-volume fluid module. Only the Coulter output based on volume measurements was used for analysis.

In Vitro Release Study

We generated release profiles for all of the formulations by incubating them in PBS buffer (pH = 7.2) at 37°C. The experiment was carried out keeping the total concentration of dicumarol in water below the solubility limit, 28 μg/mL (16). All release studies were scaled up to 5 mg dicumarol in 180 mL PBS buffer, and each group consisted of $n = 4$ samples. Mild agitation was used at the onset of these studies to create a particulate suspension. At different time points, 120 μL supernatant was obtained from each sample, placed into an Amicon (Bedford, MA) Ultrafree-MC centrifugal filter device with a nominal molecular weight cutoff of 5 kDa, and centrifuged for 5 min at 11,269 *g* to remove any residual crystallized dicumarol. Then, 100 μL of the supernatant was removed and stored at 4°C until it was analyzed, and 120 μL of fresh buffer was added back to each sample after the time points.

Animal Models

The following animal work was performed in accordance with the Principles of Laboratory Animal Care (NIH publication #85-23, revised 1985). Both female Yorkshire pigs and male Sprague–Dawley rats were used throughout the study. Pig starting weights ranged from 15 to 20 kg, and they were divided into groups of $n = 3$, 4, or 5 throughout the study, depending on availability. The groups were kept for 12–14 weeks and were administered each formulation throughout the study. Male CD rats weighing approximately 250 g were also used and were divided into groups of between 8 and 12 for each study group. The rat groups were each used for only one study and were sacrificed after the last time point.

After a fasting period of 12 h, animals were orally gavaged with microsphere formulations suspended in a solution of 1% HPMC and 1% pluronic F127. The microsphere dose

was suspended immediately before administration using bath sonication for 3 min and was administered to the stomach through a gavage tube. The suspension concentration was kept constant at 25 mg/mL, and several flushes of vehicle were administered following the dose. During administration, pigs were sedated with a combination of ketamine and medetomidine, and immediately following the procedure, the medetomidine antagonist atipamezole was given for reversal of anesthesia. Rats were anesthetized using isoflurane gas.

A control group in each species was gavaged with blank p(FA:SA) 17:83 microspheres suspended in 1% F127/1% HPMC in order to generate a baseline for the experiment. Additionally, both animal models had an IV group to which we administered dicumarol dissolved in a mixture of 50% propylene glycol, 10% ethanol, and 40% 100 mM Tris at a pH of 9.0. The IV dose of 25 mg/kg was administered to catheterized rats purchased from Zivic Miller Laboratories.

The 300-μL blood samples were obtained by transecting the tip of the tail, and subsequently at each time point the injury was irritated, and blood was collected. The following time points were generally used for blood sampling: 0, 0.25, 0.5, 0.75, 1, 2, 3, 5, 8, 11, 14, 24, 30, 35, and 48 h.

The pig group was administered 25 mg/kg dicumarol in the same IV vehicle as described previously. The dicumarol was dissolved in the vehicle at a concentration of 20 mg/mL and administered through a chronic catheter placed in the external jugular vein. Losses in the catheter were determined to be equivalent to 4% of the dose by NaOH extraction, so the effective dose delivered was 24 mg/kg. At specific time points, generally 0, 1, 2, 3, 5, 8, 11, 14, 25, 29, 36, 48, 60, 72, 84, and 96 h, blood was collected from each animal. In the pig, the heparin block was removed, 1 cc of fresh blood was collected, and another 1.5 cc heparin solution was added to the catheter. Then 300 μL of rat blood was sampled from the tail vein. The blood samples were collected in heparinized 1.5-mL siliconized microfuge tubes and centrifuged for 5 min at 11,269 *g*. Approximately 200 μL plasma was removed and stored at 4°C before being analyzed.

Dicumarol Quantification

A double extraction technique with slight modifications was used based on the method published by Nagashima *et al.* (17). A 50-μL sample of the plasma in a 15-mL Falcon tube was first acidified with 300 μL of a citrate/phosphate buffer with pH = 3.0 by shaking and allowing the mixture to interact for 5 min. Next, the dicumarol was extracted from the plasma by adding 3 mL heptane and rotating each sample end over end for 10 min. The tubes were centrifuged for 5 min at 3000 rpm, and the top heptane layer was separated and put into a new tube. Next, 1 mL of 2.5 N NaOH was added to each tube, and the mixture was again rotated end over end for 10 min. Following centrifugation at 3000 rpm for 5 min, the aqueous phase was removed, and the absorbance was read at 315 nm on a Shimadzu UV-2501 spectrophotometer. A standard curve for this assay was obtained by doping plasma obtained from control animals, from either the rat or pig, with known amounts of dicumarol in sodium hydroxide. The assay was validated with these standards to confirm the concentration using the assay. This was done with eight concentrations of dicumarol, 12.5 μg/mL through 500 μg/mL, by three operators five times each on separate days. These validations produced extremely linear standard curves that were repeatable, with an average R^2 value of 0.993 ± 0.004. The standard deviation of the assay was determined to be 4 μg/mL for concentrations between 0 and 50 μg/mL, and 8 μg/mL for concentrations between 100 and 400 μg/mL.

Bioactivity

Plasma samples taken at the T_{max} were tested for drug activity using the prothrombin time test (PTT) performed by IDEXX Veterinary Services in North Grafton, MA (18,19). Plasma was collected and submitted for testing in tubes coated with citrate.

Statistics and Pharmacokinetic Analysis

Standard errors were calculated, and a one-way ANOVA was performed, using Graphpad Prism Software. After the significance of the ANOVA had been confirmed, Dunnet's test was used using the same software with the stock dicumarol as the control group. AUC, C_{max}, and T_{max} were also calculated from the Graphpad Prism Software, which employs the trapezoidal rule for the calculation of AUC. Bioavailability was calculated using $F = (AUC_{oral}/Dose_{oral}) \times (Dose_{IV}/AUC_{IV})$.

RESULTS

Characterization of Dicumarol Formulations

Scanning electron micrographs of the dicumarol formulations are displayed in Fig. 1. Figure 1A shows the stock dicumarol as supplied by Sigma. The particulates are primarily in the range of 10–20 μm and have a cubic appearance. Spray-dried dicumarol, shown in Fig. 1B, has a round appearance and appears to be hollow. These particles are roughly 3 μm in diameter. The precipitated drug is shown in Fig. 1C. This formulation is irregular, and most of the population is in the range of 300 nm to 1 μm range. Figure 1D shows the p(FA:SA) nanospheres fabricated from the micronized formulation. Particles in this formulation are generally 1 μm in size. The loading determination of the FA:SA formulation along with the Coulter particle size data are presented in Table I.

Thermal analysis using DSC is presented in Fig. 2. ΔH is shown on the left of each melt in calories/gram. The bottom curve for the blank p(FA:SA) polyanhydride shows a variety of peaks. Between 60°C and 90°C is the trimodal peak of the polymer melt. At 275°C, the polymer and its components begin to degrade, which continues beyond 300°C. The stock dicumarol shows a distinct melt at approximately 290°C (see Fig. 2). In the p(FA:SA)/dicumarol nanosphere formulation, the melting peak is completely absent, which may indicate that a solid solution has been achieved. No other peaks were seen to indicate other polymorphs of dicumarol. Similar results were obtained by others by formation when dicumarol was mixed and lyophilized with milk, with powder diffraction indicating a solid solution (12). The spray-dried and micronized formulations both show the same drug melt at about 290°C, but they have a lower ΔH than the stock dicumarol. Because the magnitude of ΔH is proportional to the relative amount of the crystalline component, the lower ΔH could result from the quenching of the drug solution during both of

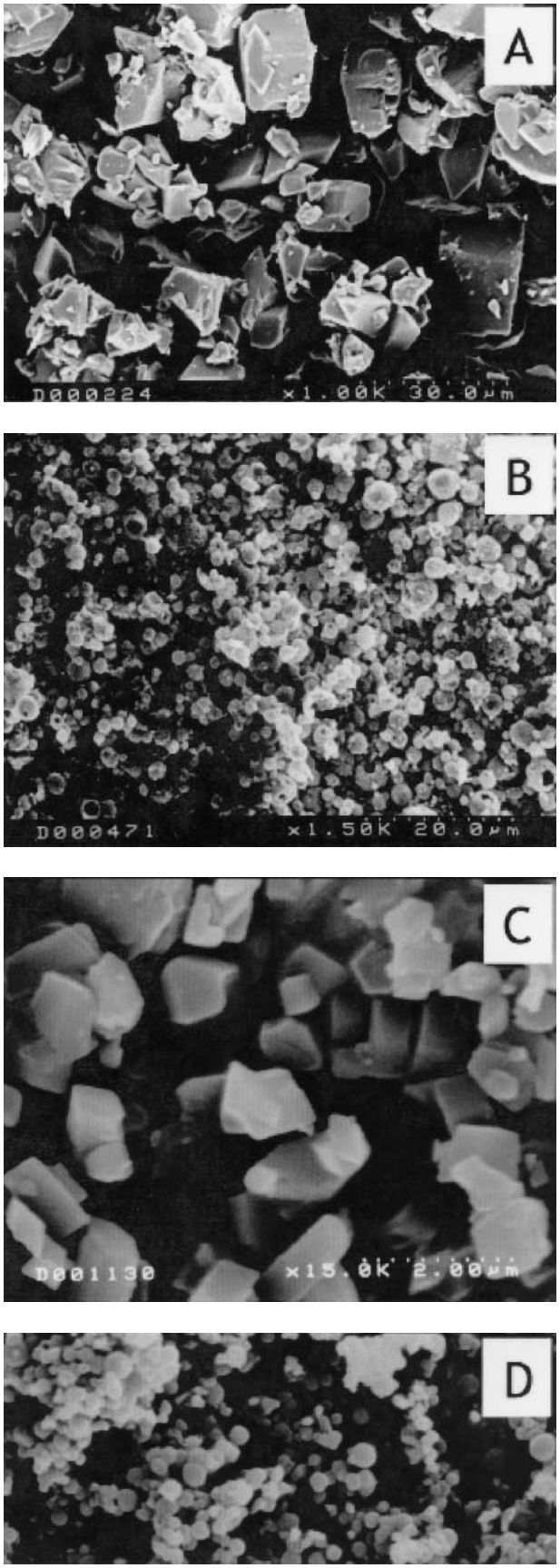

Fig. 1. Scanning electron micrographs of dicumarol formulations. (A) Stock dicumarol. (B) Spray-dried dicumarol. (C) Micronized dicumarol. (D) Dicumarol in p(FA:SA).

those processes, which could lead to decreased crystallinity. This process affected the crystallinity of the spray-dried formulation the most because the ΔH was reduced by 32%.

The dissolution rates, presented in Table I, revealed a wide range of dissolution from the different formulations tested. These rates are calculated based on the first 2 h of dissolution. The micronized formulation shows the most rapid dissolution, at 1.54 μg/mL/h, followed by the encapsulated drug at 0.44 μg/mL/h, stock dicumarol at 0.36 μg/mL/h, and the spray-dried drug at 0.18 μg/mL/h.

In Vivo Studies

Bioactivity tested positive in the prothrombin time test. Samples taken from plasma corresponding to the C_{max} all showed clotting times longer than 90 s, compared to 12 to 17 s in normal animals.

Shown in Fig. 3 are the control curves, including the IV bolus injection and oral delivery of blank p(FA:SA) nanospheres to both the rat and pig. The IV curve peaks very rapidly and shows only a downward slope indicating elimination. The blank microsphere plasma curves show that there is negligible interference of p(FA:SA) with the detection of dicumarol, as both curves for the rat and pig are extremely low and fluctuate within about 5 mg/ml of the baseline.

Drug formulations were administered to animals at a dose of 25 mg/kg. The p(FA:SA) nanospheres, however, were fed at a lower dose. Figure 4 shows the plasma curves generated from the rat experiments. All doses were 25 mg/kg except for the p(FA:SA) formulation, which was 18.2 mg/kg. The nanoparticulate drug formulation shows the highest concentration, reaching 120 μg/mL after only 3 h, followed by a continuous decrease until 60 h. The next highest concentration was achieved by the spray-dried dicumarol particles, which reached 90 μg/mL after 3 h and declined very rapidly within the next 30 h. The p(FA:SA) nanosphere formulation showed high concentrations in the blood until 60 h. This formulation reached 88 μg/mL after 6 h, decreasing to 47 μg/mL after 24 h, where it stayed for an additional 12 h. The stock dicumarol showed the lowest levels of concentration, reaching 64 μg/mL after 3 h and decreasing significantly after 15 h.

Results in the pig were very similar to those from the rat except for a more pronounced difference between formulation groups (Fig. 5). Again, all doses are 25 mg/kg except for the p(FA:SA) formulation, which was 18.2 mg/kg, and the IV dose that was administered at 24 mg/kg. The small particulate formulation shows T_{max} reaching 112 μg/mL after 5 h, and a second very small peak at 30 h that is not statistically significant. The p(FA:SA) formulation shows a more prolonged duration, with high concentrations extending to 30 h. The spray-dried dicumarol peaks at 2 h at a concentration of 86 μg/mL and rapidly decreases by 24 h. In both rats and pigs, it is clear that the nanoparticulate drug and p(FA:SA) formulation offer an advantage over the spray-dried formulation and the stock dicumarol. Pharmacokinetic analysis can be used to more efficiently compare the formulations and to draw conclusions based on both the animal model and the characteristics of the formulation.

Pharmacokinetic calculations are presented in Table II. In both cases, the p(FA:SA) nanosphere formulation shows the highest relative bioavailability, with 132% in the rat and 113% in the pig. The polymer's ability to control the release

Table I. Coulter Particle Size Analysis of Dicumarol Formulations

Formulation	Coulter particle analysis 25% <	50% <	75% <	Loading	Dissolution rate (μg/mL/h)
Stock	12.98 μm	18.95 μm	24.82 μm	100%	0.36
Spray dried	1.474 μm	3.053 μm	5.319 μm	100%	0.11
Micronized	0.433 μm	0.535 μm	0.701 μm	100%	1.54
Encapsulated in FA:SA	0.782 μm	1.414 μm	2.312 μm	31%	0.44

Note: Data reported was generated from Coulter volume calculations.

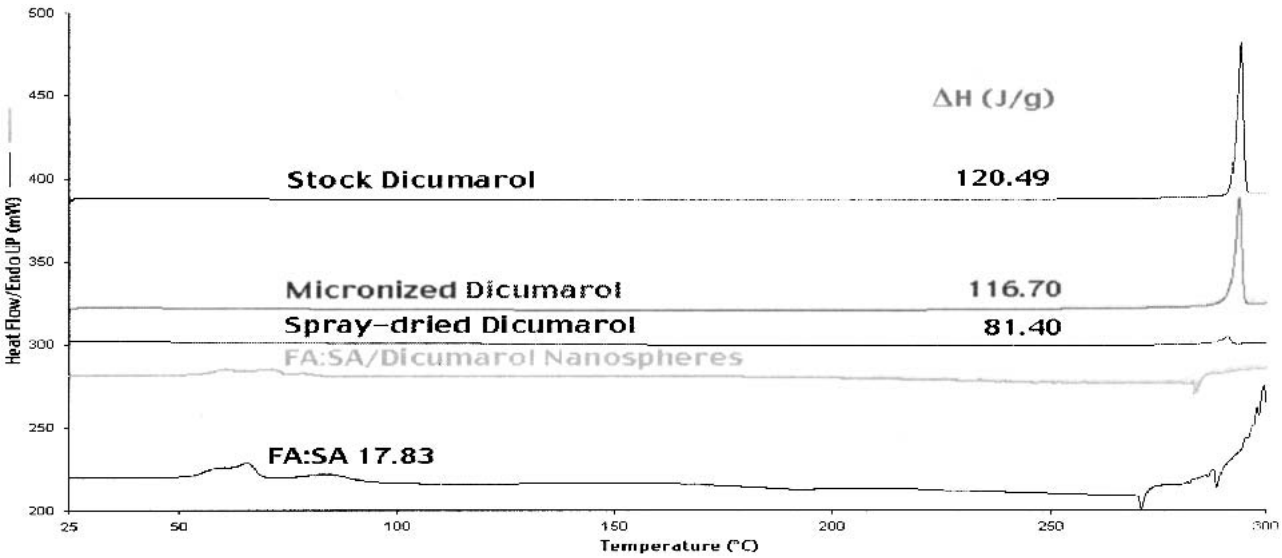

Fig. 2. DSC thermogram of dicumarol formulations.

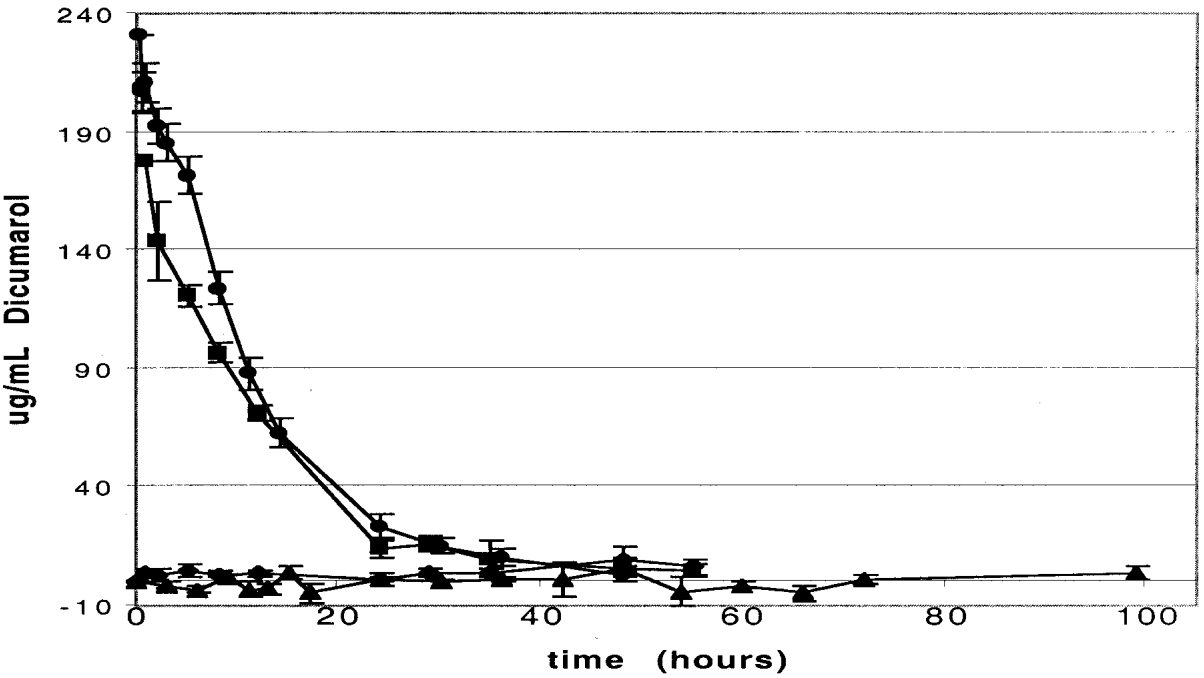

Fig. 3. Pig and rat control curves. Points represent mean ± standard error. ■, IV (pig). ●, IV (rat). ♦, Blank FA:SA microspheres (pig). ▲, Blank FA:SA microspheres (rat).

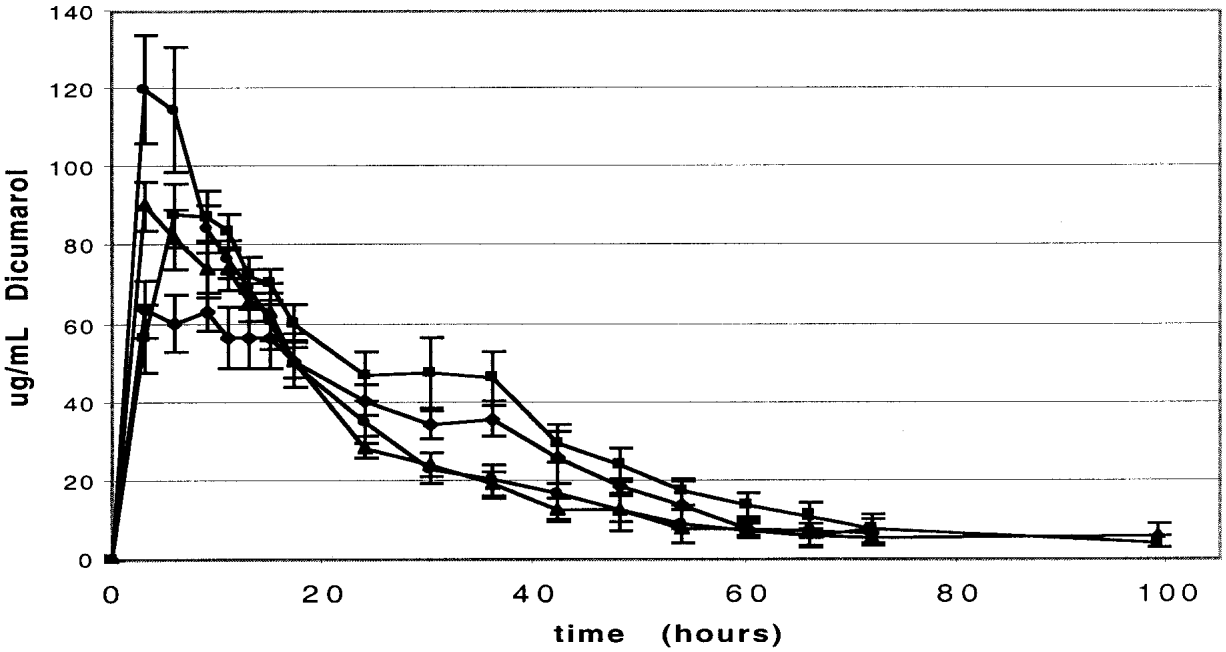

Fig. 4. Plasma curves in the rat. Points represent mean ± standard error. ●, Micronized dicumarol. ▲, Spray-dried dicumarol. ■, FA:SA dicumarol nanospheres. ♦, Stock dicumarol.

and duration is also reflected by these results. Within each species, C_{max} is among the lowest and T_{max} is the highest in the p(FA:SA) formulation. Because this system is probably a solid solution, the dissolution of the drug is totally dependent on the degradation of the polymer, which in the case of p(FA:SA) is a relatively fast surface degrading mechanism (21,22). The nanoparticulate drug also shows improved relative bioavailability over other formulations, with 100.9% in the rat and 100.0% in the pig. In this case, control of duration afforded by the polymer of the previous formulation is not seen, but instead there are an increase in C_{max} and decrease in T_{max} in both species. The spray-dried dicumarol formulation shows the lowest relative bioavailability, with only 85.3% in the rat and 58.2% in the pig. C_{max} and T_{max} are intermediate between the micronized and p(FA:SA) formulations in both the rat and pig.

Statistical analysis of the pharmacokinetic data was performed in order to compare the spray-dried, micronized, and p(FA:SA) nanosphere dicumarol formulations. A one-way ANOVA and Dunnet's test were used to compare AUCs normalized by dose. The ANOVA between all groups produced *F*-values of 6.25 in the pig and 10.27 in the rat, both of which correspond to $p < 0.05$. In the pig, Dunnet's test against the spray-dried formulation showed that $p < 0.05$ for both the

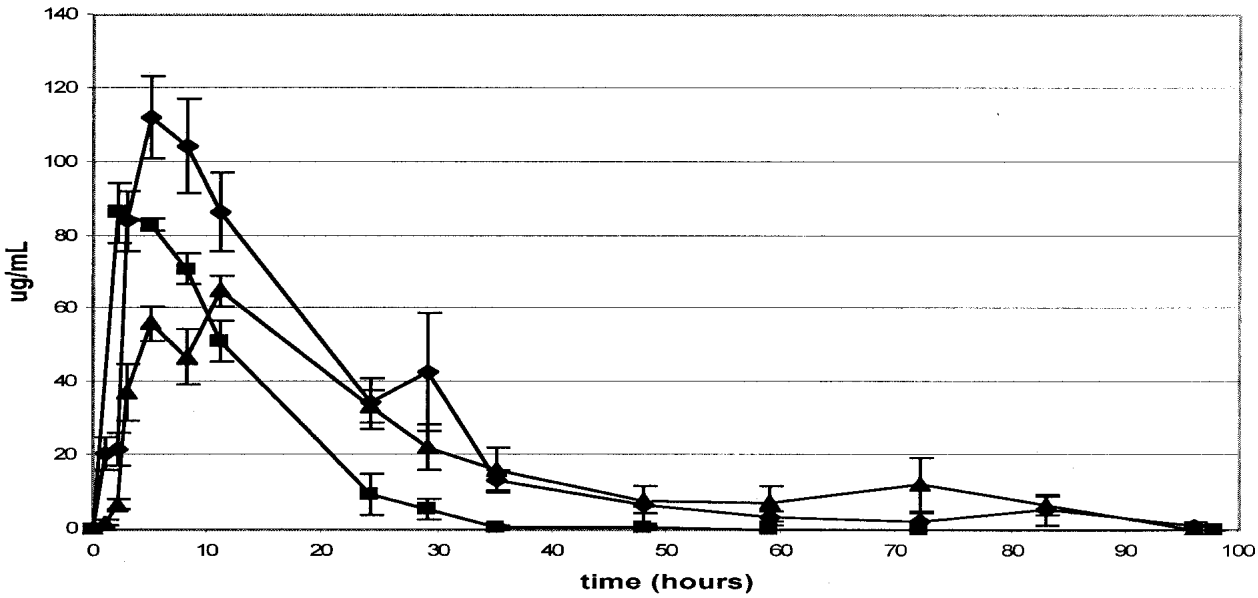

Fig. 5. Plasma curves in the pig. Points represent mean ± standard error. ■, Spray-dried dicumarol. ♦, Micronized drug. ▲, FA:SA dicumarol nanospheres.

Table II. Pharmacokinetic Calculations

Formulation	Dose (mg/kg)	C_{max} (μg/mL)	T_{max} (h)	AUC	AUC/Dose	Relative bioavailability
Rat model						
IV bolus	25	232 ± 4.5	0.25 ± 0.0	2625 ± 41	105 ± 1.6	100 ± 0.0%
Stock	25	73 ± 6.0	14 ± 2.0	2246 ± 206	90 ± 8.2	90.1 ± 2.6%
Spray dried	25	100 ± 6.0	7 ± 1.0	2238 ± 109	90 ± 4.4	85.3 ± 1.4%
Micronized drug	25	144 ± 13.0	4.2 ± 0.7	2624 ± 233	105 ± 9.3	100.0 ± 9.1%
FA:SA nanospheres	18.2	75 ± 7.7	21.1 ± 7.5	2535 ± 157	139 ± 8.6[a]	132.6 ± 10.7%[a]
Pig model						
IV bolus	24	178 ± 16.5	1.0 ± 0.0	2116 ± 155	88 ± 6.5	100 ± 0.0%
Spray dried	25	91 ± 5.0	2.8 ± 0.8	1283 ± 102	51 ± 4.1	58.2 ± 7.5%
Micronized drug	25	117 ± 13.1	6.5 ± 0.9	2366 ± 407	95 ± 16.3[a]	100.9 ± 8.9%[a]
FA:SA nanospheres	18.2	67 ± 3.4	10.4 ± 0.6	1848 ± 164	102 ± 9.0[a]	113.8 ± 14.6%[a]

[a] In the rat signifies Dunnet's test yields $p < 0.05$ for normalized AUCs against the stock drug, and in the pig against spray-dried dicumarol.

smallest microparticulate formulation and the bioadhesive formulation. In the rat, with either the stock or spray-dried as the control, $p < 0.05$ for the bioadhesive formulation.

DISCUSSION

This work has demonstrated the ability to improve the relative oral bioavailability of dicumarol by reducing particle size. Additionally, formulating nanospheres from p(FA:SA) and dicumarol, which formed a solid solution, effectively modulated pharmacokinetic parameters and also improved bioavailability. Both formulations tested positive for biologic activity using the PTT test with the C_{max} sample, and DSC analysis has shown that the crystallinity is maintained in the micronized formulation and that the p(FA:SA) formulation formed a solid solution as evidenced by the disappearance of a peak for the melt.

In vivo, relative bioavailability of a spray-dried formulation was increased by 17% in the rat and 72% in the pig by further reducing particle size. The bioadhesive p(FA:SA) formulation also improved relative bioavailability over the spray-dried drug, increasing by 55% in the rat and 96% in the pig. Additionally, the p(FA:SA) formulation prolonged T_{max} and decreased C_{max} in both species.

The enhancement offered by the p(FA:SA) formulation is caused in part by improved solubility, as shown in the dissolution studies in Table I. In a separate publication, Macheras *et al.* (12) have improved the oral delivery of dicumarol by making an amorphous mixture with milk.

In our case, the ability of the polymer to adhere to the mucosa could contribute to the observed improvement. We have previously shown the polyanhydride p(FA:SA) to be extremely bioadhesive and to reside longer in the GI tract (23–28), and in a nanosphere formulation, it has the potential to be transported into the epithelium and into the lymphatics (20). Both of these properties, bioadhesion and uptake, could also explain the enhancement seen in the p(FA:SA) formulation.

In the pig, the dicumarol formulation with the smallest particle size showed a significant improvement in relative bioavailability over the spray-dried formulation. This is probably related to the improved solubility of this formulation, shown in Table I, because the surface and composition of the drug have not been changed in any way. In the rat, it is not clear why the small-size formulation failed to show a significant enhancement over the spray-dried dicumarol. Perhaps the particles were retained in the pig for longer periods of time. Additionally, there may be a difference *in vivo* between the dissolution rates of the formulation in pigs and in rats.

The technique described to reduce particle size may be applicable to a wide range of therapeutic agents. By incorporating the bioadhesive polyanhydride, we have created a formulation with improved dissolution as well as bioadhesive properties. Future work will target other hydrophobic molecules.

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