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Research paper

Cogrinding enhances the oral bioavailability of EMD 57033, a poorly water soluble drug, in dogs

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Abstract

The oral bioavailability of EMD 57033, a calcium sensitizing agent with poor solubility, was compared in dogs using four solid dosage form formulation approaches: a physical blend of the drug with excipients, micronization of the drug, preparation of coground mixtures and spray-drying of the drug from a nanocrystalline suspension. The formulations contained generally accepted excipients such as lactose, hydroxypropylmethyl cellulose and sodium lauryl sulphate in usual quantities. Drug micronization and cogrinding was realized by a jet-milling technique. Nanoparticles were created by media milling using a bead mill. All formulations were administered orally as dry powders in hard gelatine capsules. While micronization increased the absolute bioavailability of the solid drug significantly compared to crude material (from nondetectable to 20%), cogrinding with specific excipients was able to almost double this improvement (up to 39%). With an absolute bioavailability of 26%, spray-dried nanoparticular EMD 57033 failed to show the superior bioavailability that had been anticipated from *in vitro* data. The control solution prepared with cyclodextrin was shown to have an absolute bioavailability of 57% (vs. i.v. infusion). It was concluded that cogrinding can be a useful tool to improve the bioavailability of poorly soluble drugs from a solid dosage form format.

Keywords: Bioavailability; Cogrinding; *In vitro–in vivo* correlation; Jet-milling; Dissolution rate enhancement; Micronization; Particle size reduction; Poorly soluble drugs; Spray-drying

1. Introduction

During the last decade there has been an increasing trend in pharmaceutical research to produce drug candidates that exhibit high lipophilicity and poor water solubility [1]. Such physicochemical characteristics lead to problematic biopharmaceutical properties, which may diminish or even preclude success in the clinic [2].

The aim of the present canine study was to compare the effect of several processing techniques on the bioavailability of a poorly soluble drug in order to identify promising approaches for development of solid oral dosage forms for drugs with problematic dissolution kinetics. EMD 57033, a calcium sensitizing agent [3], was chosen as a typical example of a poorly soluble drug (aqueous solubility 5 μg/ml at 37 °C). The bioavailabilities of a physical mixture of EMD 57033 with lactose, a physical mixture of micronized drug with lactose, a coground mixture with lactose, a coground mixture with hydroxypropylmethyl cellulose as well as a formulation of EMD 57033 with lactose and sodium lauryl sulphate (SLS) prepared by spray-drying from a nanoparticulate drug suspension were compared.

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All formulations were administered orally to dogs as dry powders in hard gelatine capsules. Hydroxypropyl-β-cyclodextrin solutions served as oral and intravenous controls. The *in vivo* results were then compared to *in vitro* dissolution studies of the formulations in biorelevant media [4] using established *in vitro–in vivo* correlation (IVIVC) techniques.

2. Materials and methods

2.1. Chemicals

EMD 57033 (see Fig. 1) was a development candidate from Merck KGaA (Darmstadt, Germany). The drug was synthesized by alkaline hydrolysis of its chemically modified prodrug EMD 82571 (lot 02/HH/31). Lactose monohydrate, HPMC 2910/5 and SLS were from Merck KGaA (Darmstadt, Germany). Sodium taurocholate was obtained from Prodotti Chimici E Alimentari S.P.A. (Basaluzzo, Italy). Egg-phosphatidylcholine, Lipoid E PC, was purchased from Lipoid GmbH (Ludwigshafen, Germany). Hydroxypropyl-β-cyclodextrin was purchased from Sigma–Aldrich (Steinheim, Germany). All other chemicals used were of HPLC grade or analytical grade.

2.2. Preparation of the test formulations

Formulation A was a dry powder mixture and was prepared by physically blending EMD 57033 (10%) with lactose (90%), and then manually filling the blend into Coni-Snap Supro A hard gelatine capsules (Capsugel, Belgium). Formulations B through D were prepared by milling either the drug substance alone or as a physical mixture with specific excipients using an Alpine 50 AS jet-mill (Hosokawa Alpine AG, Germany) operated at 5 bar air pressure and a feed rate of 0.5–1.0 g/min. The milled powder was then manually filled into Coni-Snap Supro A hard gelatine capsules, after blending with lactose, if necessary, to obtain a final drug concentration of 10%. Correspondingly, Formulation B contained jet-milled drug in a physical mixture with 90% lactose. Formulation C comprised a coground mixture of EMD 57033 with lactose in a 10:90 ratio. Formulation D consisted of EMD 57033 coground with hydroxypropylmethyl cellulose in a ratio of 50:50, which was then physically blended with 80% lactose. Homogeneity of the mixtures was investigated by quantitative HPLC determination of the drug content after accurate weighing of aliquots of powder, dissolving and diluting with mobile phase ($n = 3$).

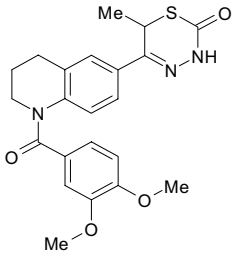

Fig. 1. Chemical structure of EMD 57033 (MW = 425.5).

Formulation E, a nanoparticulate dispersion of EMD 57033, was prepared by a media milling process using a Dyno Mill (Willy A. Bachofen AG Maschinenfabrik, Switzerland) operated in the circulation mode. A 300 ml cylindrical steel vessel with inside coating was filled with 0.1 mm grinding spheres to approximately 85% of the volume. A 600 ml suspension containing 30 g EMD 57033, 30 g lactose and 3 g SLS in water was pre-treated in an Ultra-Turrax at 20,500 min^{-1} before processing in the mill for 90 min. The resulting nanoparticulate suspension was diluted with 300 ml water immediately prior to feeding to the 0.7 mm pneumatic spray nozzle of a Büchi Mini Spray Dryer B-191 (Büchi Labortechnik AG, Switzerland). The mill was kept operating during the spray-drying process in order to maintain homogeneity of the suspension. The spray dryer was operated at 6 bar air pressure, 11 ml/min pump speed, 600 l/h air flow rate, 80% aspirator level and 150 °C inlet temperature.

2.3. Particle size measurement

Particle size was determined by laser light diffraction using a Malvern Mastersizer 2000 (Malvern Instruments, Germany) including a Scirocco 2000 module for dry measurement purposes operating at 3.0 bar air pressure for dispersion. Data were evaluated with Malvern software version 4.0 using the Fraunhofer approximation as the evaluation algorithm [5].

2.4. HPLC analysis

The system consisted of a Merck Hitachi pump L-6200A, a Merck Column Thermostat T-6300 operating at 36 °C, a Merck Hitachi Interface D-6000A, a Merck Hitachi UV–Vis Detector L-4250 and a Merck Hitachi Autosampler AS-4000A. Data acquisition and evaluation was performed with Merck Hitachi D-7000 Chromatography Data Station Software version 4.0. Using a LiChrospher 60 RP select B 125-3 (5 μm) column and a mobile phase consisting of 65% of pure water and 35% of acetonitrile at a flow rate of 1 ml/min, EMD 57033 was eluted at approximately 5 min. The detection wavelength was set at 321 nm.

2.5. Solid state characterization by X-ray diffraction

Powder X-ray patterns were recorded using a Bruker AXS diffractometer (Bruker AXS GmbH, Germany) with a PSD-50M detector and EVA Application Software version 6. Measurements were performed with a Cu Kα radiation source at 40 kV voltage, 30 mA current and a maximum scanning speed of 2°/min.

2.6. Dissolution testing

Release from the capsules was determined in a calibrated USP XXVIII apparatus 2 (paddle method) in 900 ml medium using a PharmaTest dissolution tester (Type PTWS, Pharmatest, Germany) operating at 75 rpm and 37 °C. Helix sinkers (11/31, 8/23, Sotex GmbH, Germany) were used to prevent floating of the capsules. Each 3 ml sample was immediately filtered through a 0.22 μm membrane filter and appropriately diluted with HPLC mobile phase prior to analysis.

3. Canine studies

3.1. Animals

Four female mongrel dogs, aged 5–6 years and weighing between 27 and 33 kg, were used for the study. The study was performed at an animal facility operated according to European Union regulations for the maintenance and experimentation on animals and the study was approved by the Veterinary Directorate of the Municipality of Athens, Greece.

3.2. Preparation of drug solutions for oral and intravenous administration

The oral solution consisted of 250 ml of an aqueous solution of a one-to-one complex of EMD 57033 with HP-β-CD, corresponding to 30 mg of EMD 57033 (Formulation F). The intravenous dosage form consisted of 80 ml of a 5% dextrose solution containing 20 mg of EMD 57033 complexed with HP-β-CD (Formulation G). Both dosage forms were prepared on the afternoon prior to administration and the concentration verified by HPLC.

3.3. Study protocol

The five solid formulations (A–E) in hard gelatine capsules as well as the drug solutions for oral and intravenous administrations (F and G) were tested in a completely randomized crossover design. Each oral single dose corresponded to 30 mg of EMD 57033, except for the i.v. dose, which was 20 mg. The dogs were fasted for at least 16 h before each administration, but had access to water. Test capsules were given with 250 ml of tap water orally via an orogastric tube. The oral HP-β-CD solution was administered similarly. Intravenous infusion of Formulation G was started immediately after administration of 250 ml of water via the orogastric tube, and took place over 1.16 h at an infusion rate of approximately 1.2 ml/min.

Blood samples were collected immediately prior to and at 15, 30, 45, 60, 90, 120, 180, 240, 360 and 480 min after administration (for the i.v. infusion times were calculated from the start of the infusion). After immediate centrifugation, plasma was stored at −20 °C until assayed for drug concentration per HPLC. Six hours post-administration the dog was offered a standard meal (150 g pellets and 200 ml tap water). Eight hours after dosing, the catheter was removed and the dog returned to her cage, where she was allowed to eat and drink ad libitum. The washout period between the intervals was at least 7 days for each dog.

3.4. Quantification of EMD 57033 in plasma samples

EMD 57033 in the sampled plasma was assayed by HPLC using a reversed-phase Hypersil BDS C_{18}column (150 × 4.6 mm, 5 μm particle size) equipped with a precolumn Hypersil BDS-C_{18} (10 × 4 mm, 5 μm particle size). The chromatographic system consisted of a Spectra System P1000 pump, a Spectra System UV 2000 absorbance detector and an autosampler AS 3000. The system was controlled by a Spectra System Controller SN 4000 and the software package Chromquest (Thermoquest Inc., San Jose, USA). The mobile phase was composed of acetonitrile and water (40:60 v/v). The flow rate was set to 1 ml/min, the injection volume was 75 μl. EMD 57033 and the internal standard, EMD 54616 (supplied by Merck KGaA, Germany), were eluted at approximately 6.6 and 11.5 min, respectively, at the detection wavelength of 321 nm.

Plasma samples (500 μl) were transferred to a microcentrifuge tube containing 50 μl of a working solution of the internal standard (1 μg/ml) and 450 μl of acetonitrile was added. After vortexing for 1 min, the samples were centrifuged (Hettich Universal 32R, Tuttlingen, Germany) for 15 min at 11000 rpm/10 °C. The clear supernatant was then injected into the HPLC system. Calibration was performed in plasma and the concentration range for EMD 57033 was 10–250 ng/ml. The limit of quantification (LOQ) was 10 ng/ml.

3.5. Pharmacokinetic calculations

The maximum plasma concentration (C_{max}) and the time at which it occurred (t_{max}) were observed from the individual subject plasma concentration time profiles. The area under the plasma concentration vs. time curve from time zero to infinity (AUC) was calculated according to the trapezoidal rule. Results are presented as mean values of the parameters and their standard deviation (SD).

4. Results and discussion

4.1. Results

Table 1 summarizes the seven formulations prepared and the rationale for their inclusion in the pharmacokinetic study. Table 2 shows the particle size distribution of the five test formulations. Fig. 2 illustrates the *in vitro* dissolution profiles of the test formulations in 0.12% SLS solution. The pharmacokinetic results are given in Table 3, the plasma concentration vs. time curves for the four dogs are shown in Fig. 3 and the absolute and relative bioavailability results of EMD 57033 are listed in Table 4. Fig. 4

Table 1
Formulations of EMD 57033 administered to four female dogs in the pharmacokinetic study

Formulation	Composition	Rationale for selection	*In vitro* dissolution
A	Physical mixture of: 10% coarse EMD 57033 90% lactose	To demonstrate the baseline situation	Very poor dissolution rate
B	Physical mixture of: 10% micronized EMD 57033 90% lactose	To demonstrate the effect of particle size reduction	dissolution rate increased
C	Coground mixture of: 10% EMD 57033 90% lactose	To compare the cogrinding technique with drug micronization	Rapid, but unstable, supersaturation
D	Coground mixture of: 50% EMD 57033/50% HPMC in a physical mixture with 80% lactose	To compare the addition of a polymer in coground mixtures	Supersaturation more stable than for Formulation C
E	With lactose and SLS spray-dried EMD 57033 from a nanoparticulate suspension	To demonstrate the effect of a nanosizing/spray-drying technique	Stable supersaturation
F	EMD 57033-HP-β-CD	To show maximal oral bioavailability	–
G	EMD 57033-HP-β-CD	Parenteral control, for calculation of absolute bioavailability	–

Table 2
Particle size distribution of EMD 57033 and its formulations, expressed in volume-based *d*-values that correspond to 10%, 50% and 90% of the total particle population, respectively

	d(0.10) (μm)	*d*(0.50) (μm)	*d*(0.90) (μm)
Unprocessed drug	16.4	227.6	432.9
Micronized drug	0.7	2.2	6.9
Cogrind with 90% lactose	0.6	2.9	7.5
Cogrind with 50% HPMC	0.8	4.4	45.0
Spray-dried formulation	1.3	4.5	9.2

depicts the correlations of the *in vitro* dissolution studies to the *in vivo* results (IVIVC) using the Level C approach. For Formulation A, the unmilled drug, plasma concentrations were all below the limit of quantification.

4.2. Discussion

Many approaches have been developed to improve solubility and to enhance the dissolution rate of poorly soluble drugs. Physical modifications often aim to increase the surface area, solubility and wettability of the powder particles and are therefore focused on particle size reduction or generation of amorphous states [6,7]. In some cases, both conversions may occur simultaneously (e.g. during spray-drying). Although the increase of bioavailability after micronization of drugs is well known (e.g. danazol [8], progesterone [9], digoxin [10]), in some cases a decrease in particle size may lead to poor wettability and flow properties [11,12]. Taking particle size reduction the next step, nanosizing can be achieved by precipitation or by milling. The latter requires special techniques such as bead milling [13,14] or high pressure homogenization [15]. In order to obtain a dry form, further pharmaceutical operations are required (e.g. lyophilisation, spray-drying).

Cogrinding processes are comparatively seldom described in the literature and have often employed large quantities of water soluble polymers as dispersion carriers [16,17]. The improvement of the dissolution of poorly soluble drugs by cogrinding or comelting with surfactants such as sodium lauryl sulphate (SLS) or sodium desoxych-

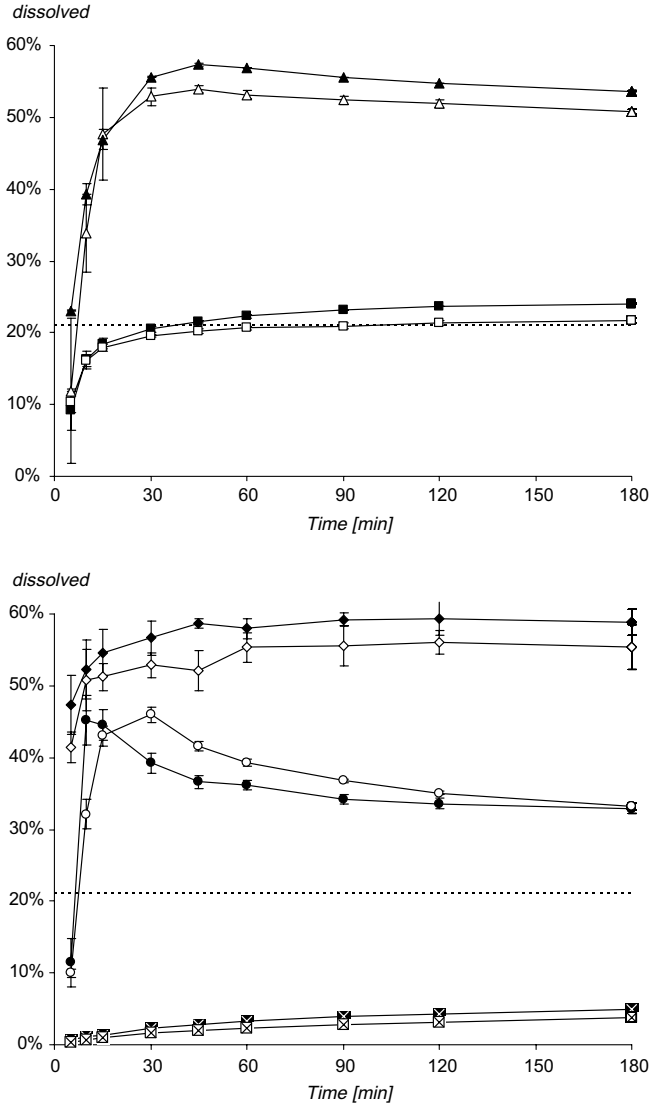

Fig. 2. Dissolution profiles of 30 mg EMD 57033 in Formulation A (⊠), Formulation B (■), Formulation C (●), Formulation D (▲) and Formulation E (♦) in 0.12% SLS solution ($n = 3, \pm$ SD). Open symbols represent the dissolution profiles of the corresponding formulations after a storage period of 16 months (Formulation A), 13 months (Formulations B–D) and 2 months (Formulation E) at ambient temperature. Dotted line indicates the EMD 57033 solubility limit.

Table 3
Pharmacokinetic results (C_{max} in ng/ml, t_{max} in min, AUC in ng h/ml) for EMD 57033 in seven formulations after administration to four healthy, female dogs

Formulation		Dog 1	Dog 2	Dog 3	Dog 4	Mean ± SD
A	C_{max}	[a]	[a]	[a]	[a]	[a]
	t_{max}	[a]	[a]	[a]	[a]	[a]
	AUC	[a]	[a]	[a]	[a]	[a]
B	C_{max}	25.8	17.2	17.9	33.42	23.6 ± 6.6
	t_{max}	90	180	90	120	120.0 ± 37.2
	AUC	99.7	77.3	65.0	177.1	104.8 ± 44.0
C	C_{max}	77.1	25.0	24.6	33.2	40.0 ± 25.2
	t_{max}	60	90	120	90	90.0 ± 21.6
	AUC	323.4	103.7	108.2	113.3	162.2 ± 92.5
D	C_{max}	76.2	48.8	45.3	64.4	58.7 ± 14.1
	t_{max}	180	120	90	60	112.5 ± 43.8
	AUC	290.0	165.8	124.2	201.8	195.5 ± 60.6
E	C_{max}	56.4	23.7	18.1	42.7	35.2 ± 17.6
	t_{max}	120	180	180	180	165.0 ± 26.4
	AUC	233.2	75.8	71.4	171.6	138.0 ± 67.6
F	C_{max}	158.8	87.5	101.5	101.3	112.3 ± 31.4
	t_{max}	15	30	30	30	26.3 ± 06.6
	AUC	322.2	270.4	255.7	287.1	283.9 ± 25.6
G	C_{max}	169.3	118.9	123.0	249.5	165.2 ± 61.1
	t_{max}	45	45	60	90	56.3 ± 19.7
	AUC	542.6	400.5	470.9	595.6	502.4 ± 75.4

[a] No levels of EMD 57033 were detected in any samples after administration of Formulation A.

olate [18] or sugars [19] has also been investigated, but in these cases amorphous states of the drugs were generated. Spray-drying is another technique known to produce amorphous material due to rapid solvent evaporation [20]. Although conversion to the amorphous state can markedly improve solubility and dissolution characteristics, the amorphous state may revert to a lower energy state, typically crystalline, form during storage (e.g. indomethacin). Unfortunately the time-frame of such conversions is not easy to predict. A formulation which provides the drug in a rapidly dissolving, yet crystalline, form would therefore represent an optimal solid dosage form for p.o. administration.

EMD 57033 is considered a Biopharmaceutics Classification System Class II drug and is known to be poorly bioavailable after oral administration due to inappropriately slow dissolution from the dosage form [21]. Since it is a neutral substance and its solubility is only modestly increased in the presence of bile salts, it was considered a suitable candidate to investigate the effects of physical modifications on bioavailability. Further, it had been established that EMD 57033 remains crystalline after micronization and cogrinding with numerous excipients by jet-milling [22].

Formulation A showed no oral bioavailability after oral administration: no concentrations could be quantified in plasma. As corresponding *in vitro* dissolution studies revealed dissolution of less than 5% after 180 min (Fig. 2, lower panel), it can be assumed that poor dissolution is a primary cause of poor bioavailability.

Micronization (Formulation B) increased the absolute bioavailability of EMD 57033 to 20%. The particle size reduction to fine drug crystals by jet-milling (Table 2) improved the dissolution rate and hence the bioavailability of this poorly soluble (Class II) drug. Fig. 2 (upper panel) indicates a commensurate improvement in the *in vitro* dissolution behaviour (20% in 180 min).

Although the quantitative composition of Formulation C was identical to Formulation A and B, the absolute oral bioavailability increased to 32%, indicating the superiority of the cogrinding technique to simple drug micronization. Previously it was shown that this improvement was not caused by conversion of EMD 57033 to the amorphous state [23]. Nor could the result be explained by a reduction in particle size (Table 2). On the other hand, the *in vitro* dissolution profile exhibited an initial supersaturation (Fig. 2, lower panel), which would provide a higher driving force for the absorption.

The absolute bioavailability of Formulation D was 39%, the best bioavailability of the solid formulations tested. For comparison, it was demonstrated in previous work that lipid formulations of EMD 57033 prepared with Gélucire 44/14 and Vitamin E TPGS could enhance the bioavailability of the drug to 26% and 48%, respectively [21]. However, both lipid formulations contained high amounts of solubilising agents such as Soluphor P or Labrafil M 2130 CS and the manufacturing process required elevated temperatures. Corresponding to the enhanced bioavailability of the HPMC cogrind, a distinct and sustained supersaturation was observed in *in vitro* dissolution studies (Fig. 2, lower

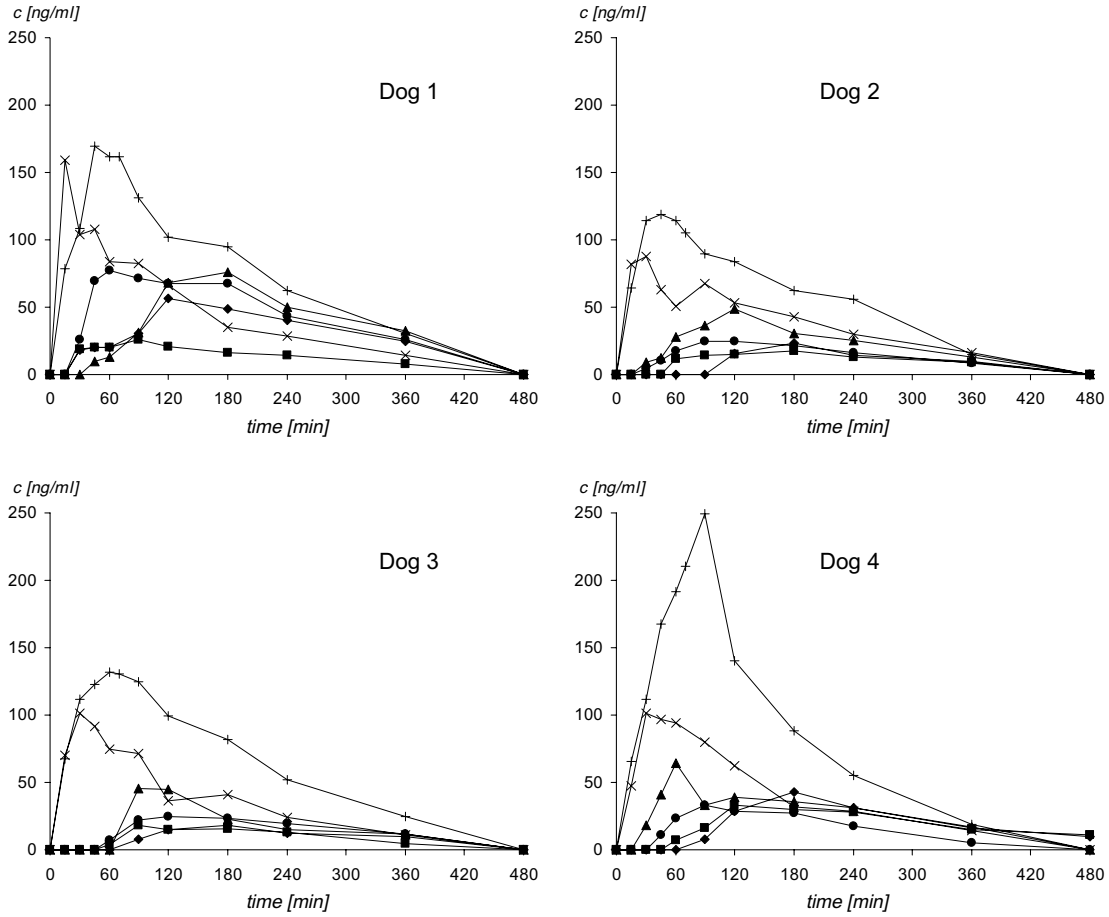

Fig. 3. Plasma concentration vs. time curves in the four dogs after administration of Formulation B (■), Formulation C (●), Formulation D (▲), Formulation E (♦), the oral solution (×) and the intravenous solution (+). Formulation A is not shown as no plasma concentrations were quantifiable in any of the dogs for this formulation.

Table 4
Absolute bioavailability (BA) and relative BA vs. oral solution (Formulation F) from five solid formulations of EMD 57033

Formulation	Absolute BA					Relative BA
	Dog 1	Dog 2	Dog 3	Dog 4	Mean ± SD	Mean ± SD
A	0.00	0.00	0.00	0.00	0.00	0.00
B	0.18	0.19	0.14	0.30	0.20 ± 0.06	0.37 ± 0.15
C	0.60	0.26	0.23	0.19	0.32 ± 0.16	0.55 ± 0.26
D	0.53	0.41	0.26	0.34	0.39 ± 0.10	0.68 ± 0.15
E	0.43	0.19	0.15	0.29	0.26 ± 0.11	0.47 ± 0.20
F	0.59	0.68	0.54	0.48	0.57 ± 0.07	–

panel). Analogous to Formulation C, this improvement was not caused by either reduction in particle size or conversion to an amorphous state. The apparently high particle size of Formulation D (Table 2) reflects the particle size of HPMC, which is resistant to particle size reduction in the jet-mill.

The absolute bioavailability of Formulation E was only 26%, despite having the highest release *in vitro* (Fig. 2, upper panel). It is hypothesized that the 0.12% SLS solution used as the dissolution medium together with the SLS in the formulation may have resulted in solubilisation effects (by exceeding the CMC in hydrodynamic boundary layer) which hindered recrystallization from the supersaturated solution. As SLS is not a natural component of the GI fluids, the effect would not have been possible *in vivo*. Since strong crystalline peaks of EMD 57033 were observed by X-ray diffraction (Fig. 5), it can be assumed that the drug remained mostly crystalline. The particle size of the spray-dried powder (Table 2) was similar to that of the micronized drug. This is probably at least partly attributable to the nanosized drug being embedded in the hydrophilic excipients during the spray-drying process, although some agglomeration of the nanosized drug may have occurred during processing.

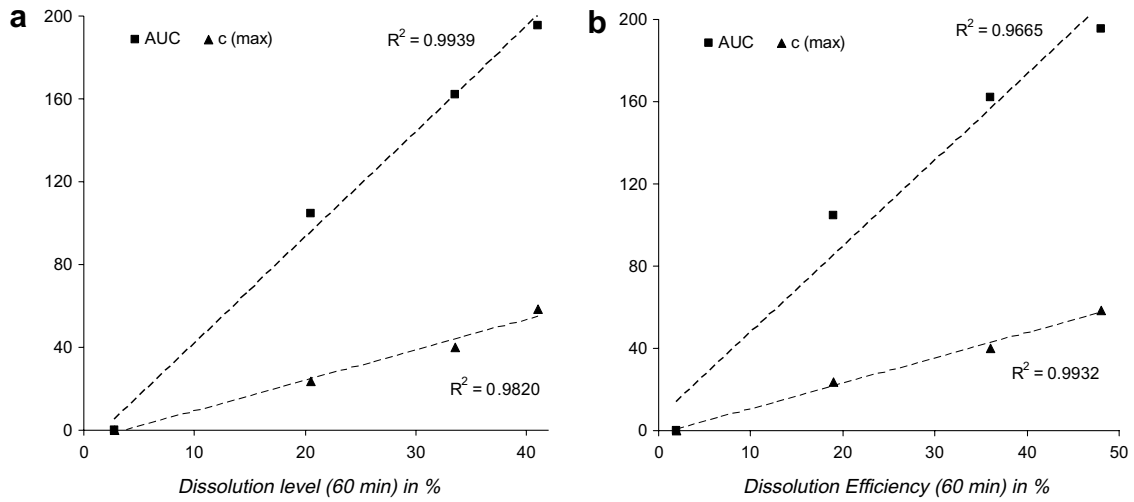

Fig. 4. Level C correlation of Formulation A, B, C and D using the *in vitro* correlation parameters (a) % dissolved at 60 min and (b) dissolution efficiency at 60 min.

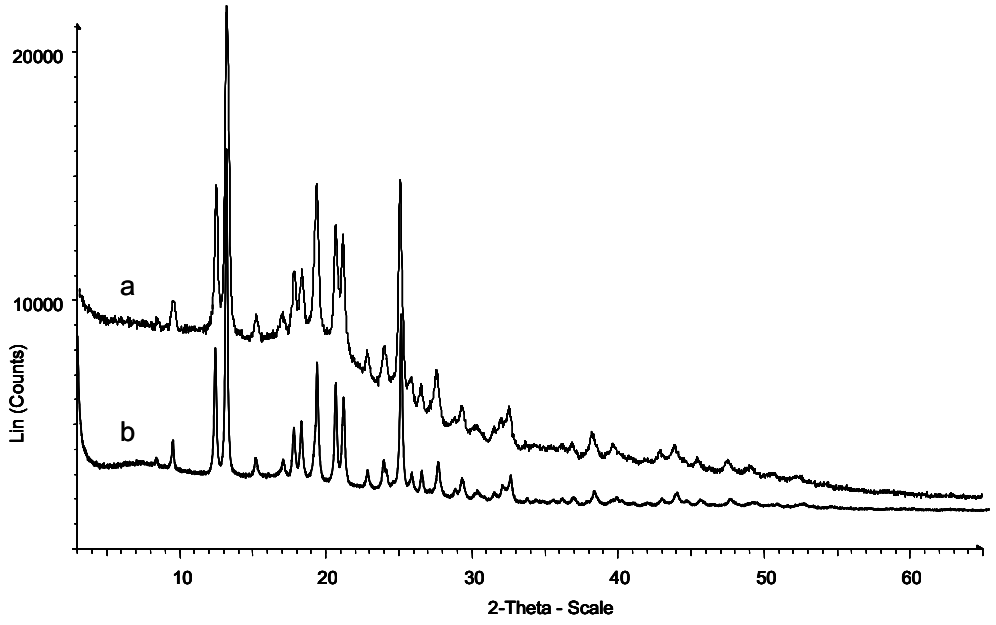

Fig. 5. X-ray analysis of Formulation E (a) and pure EMD 57033 (b).

The absolute oral bioavailability of EMD 57033 from Formulation F was shown to be 57%. This represents the upper bound of bioavailability achievable with an oral dosage form. The relative bioavailability of Formulation D to the cyclodextrin solution was 68%, indicating that most of the dissolution related limitations to absorption could be overcome with the cogrinding technique.

The stability of the dissolution profiles of the formulations is illustrated in Fig. 2. The powders were stored, protected from light, in tightly closed HDPE (high density polyethylene) bottles at ambient temperature. The storage period of up to 16 months did not affect the dissolution behaviour: similar profiles, according to the f_2 comparison procedure, were observed ($f_2 \geqslant 63$). The stability in the dissolution profiles with time is likely a result of the crystalline state of EMD 57033 in the formulations.

4.3. Level C correlation

The parameters "% dissolved at 60 min" and "dissolution efficiency at 60 min" proved to be useful for *in vitro–in vivo* correlation. The dissolution efficiency can be calculated by Eq. (1) [24]:

$$\text{D.E.} = \frac{\int_0^t y\mathrm{d}t}{y_{100}t} \tag{1}$$

where y is the percentage of drug dissolved at time point t.

Fig. 4 shows the Level C correlation between the dissolution level and the dissolution efficiency with AUC and C_{max} for Formulations A to D. In all cases, correlations resulted in linear relationships with $r^2 \geqslant 0.97$. Formulation E was excluded from the correlation as it was prepared by a completely different manufacturing process and did not fit the correlation. It is hypothesized that the drug quickly recrystallized from the (supersaturated) solution generated *in vivo*, leading to a lower oral bioavailability of Formulation E than would have been expected from the *in vitro* performance. This hypothesis is supported by the late t_{max} values observed for Formulation E.

5. Conclusion

It was demonstrated in dogs that cogrinding EMD 57033, a poorly soluble drug, with lactose or polymers increased its oral bioavailability to a far greater extent than micronization. Further, attainment of a stable supersaturation *in vitro* using a polymer (HPMC) as the cogrinding excipient appeared to be associated with better performance in the canine pharmacokinetic studies. Level C correlations with either AUC or C_{max} as the *in vivo* parameter and either dissolution after 60 min or dissolution efficiency as the *in vitro* parameter indicated a successful *in vitro–in vivo* correlation for all milled formulations, suggesting that improvements in bioavailability by cogrinding can be predicted with dissolution tests. While the canine results are very encouraging, it must be remembered that due to differences in the physiology of the gastrointestinal tract between man and dog the results may not be directly transferable to humans [25–29].

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