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Evaluation of emulsifiable glasses for the oral administration of cyclosporin in beagle dogs

Christopher J.H. Porter*, Susan A. Charman, Rachel D. Williams, Margarita V. Bakalova, William N. Charman

Department of Pharmaceutics, Victorian College of Pharmacy, Monash University, Parkville, Victoria 3052, Australia

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Abstract

Solid state emulsifiable glasses have been proposed as delivery systems for poorly water soluble drugs. This study assessed the utility of the emulsifiable glass (EG) technology for the oral delivery of cyclosporin. EG formulations were prepared, evaluated in vitro, and the bioavailability assessed in beagle dogs. Although the standard EG formulations (i.e. containing no surfactant) produced a dispersed phase upon reconstitution, significant quantities of residual oil were present within these systems. The absolute bioavailability of cyclosporin after administration of an EG cyclosporin formulation (12.5 mg dose) was compared with a 25 mg Sandimmun® capsule and a 25 mg surfactant-based self-emulsifying lipid formulation (SEDDS) in a randomized cross-over study conducted in four beagle dogs. The absolute bioavailability and the major pharmacokinetic parameters of cyclosporin were similar for the three oral formulations. Subsequently, a surfactant enhanced emulsifiable glass (SEEG) was formulated which offered the following advantages over the standard EG systems: (i) rapid, efficient and complete emulsification, (ii) a four-fold increase in drug loading capacity, and (iii) a two-fold decrease in processing time. The relative bioavailability and pharmacokinetic characteristics of the SEEG formulation were evaluated relative to Sandimmun® in a two-way crossover in four beagle dogs. There were no significant differences in either the major pharmacokinetic parameters or the relative bioavailability of the two formulations. Comparing the two studies, there was significantly less variability in the blood cyclosporin profiles after administration of the SEEG formulation than after administration of the standard EG formulation. These studies demonstrate the utility of EG technology for the oral delivery of cyclosporin, and develop the technology to include surfactant enhanced systems which offer improved characteristics.

Keywords: Emulsifiable glasses; Cyclosporin; Bioavailability; Self-emulsifying

* Corresponding author. Tel.: +61 3 99039649; fax: +61 3 99039583; e-mail: Chris.Porter@vcp.monash.edu.au

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1. Introduction

The oral administration of poorly water soluble drugs presents many formulation and bioavailability-related problems. The major biopharmaceutic limitations include low bioavailability, limited dose proportionality and highly variable systemic plasma drug concentrations (Welling, 1989; Charman et al., 1993a).

The bioavailability of poorly water soluble drugs can often be enhanced by co-administration of an appropriate lipid. This may be achieved by administering the compound with food or by formulating the drug as a dispersable lipid solution or emulsion (Winstanley and Orme, 1989; Welling, 1989; Charman et al., 1993b). While dispersed lipid or emulsion formulations may often improve bioavailability, these approaches have found limited clinical utility due to volume and taste constraints, and the limited physical stability of emulsions.

Shively and Myers have described a solid state, lipid-based emulsifiable glass (EG) system which, upon addition of water, was claimed to spontaneously form an o/w emulsion in the absence of traditional surfactants (Myers and Shively, 1992; Shively, 1993; Shively and Myers, 1993). The anticipated advantages of solid EG formulations include the ability for dosing as a solid (when filled into hard gelatin capsules), thereby increasing acceptability of the lipid containing formulation, and avoidance of the physical stability limitations of liquid emulsions. Consequently, EG systems may provide an alternative and convenient approach for the delivery of poorly water soluble drugs which benefit from the co-administration of lipid.

The present study was undertaken to evaluate the utility of an EG formulation for the oral delivery of cyclosporin, a model lipophilic drug. The study involved the development and in vitro assessment of a prototype cyclosporin EG formulation, followed by in vivo evaluation. The bioavailability study was a four-way, randomized, cross-over study conducted in four beagle dogs. The treatment legs included the commercially available intravenous and oral formulations (Sandimmun®), the prototype EG formulation, and a liquid self-emulsifying drug delivery system (SEDDS). The synthetic surfactant-based SEDDS formulation was included as a positive comparator as similar systems have previously been shown to exhibit efficient self-emulsification properties (Pouton, 1985; Charman et al., 1992). Although the bioavailability data confirmed the potential utility of the EG systems, the formulation limitations which became evident were the low and variable extents of drug incorporation and incomplete emulsification.

Further formulation work was undertaken to extend the EG concept to surfactant-enhanced emulsifiable glass (SEEG) formulations. The SEEG formulations exhibited marked improvements in drug loading capacity, redispersion characteristics (ease of reconstitution, smaller particle size) and ease of processing. A subsequent study was undertaken to assess the in vivo performance of the candidate SEEG formulation. The bioavailability study was performed as a two way cross-over in beagle dogs using the commercial oral formulation as the comparator. The results indicate that the developed SEEG formulation offers advantages over the standard EG systems, and provides a potential approach for administering lipophilic drugs whose bioavailability may benefit when co-administered with a dispersed lipid.

2. Methods

2.1. Chemicals

Cyclosporin USP (Apotex, Canada), olive oil USP (Faulding Pharmaceuticals, Australia), AR grade sucrose (Ajax Chemicals, Australia), polyoxyethylene glycerol trioleate (Tagat TO, Goldschmidt AG, Germany), Miglyol 812 (fractionated medium chain triglyceride, R.P. Scherer, Australia), peanut oil, sorbitan monooleate (Span 80), and polyoxyethylene sorbitan monooleate (Tween 80) (Sigma Chemicals, St. Louis, MO) were used as supplied. Sandimmun® concentrate for intravenous (i.v.) infusion (250 mg/5 ml) and Sandimmun® oral capsules (25 mg) were obtained from Sandoz Pharma (Australia). Sterile normal

saline was obtained from Abbott Hospital Products (Australia). Acetonitrile (ACN) was high performance liquid chromatographt (HPLC) grade and all other solvents and reagents were of at least analytical reagent grade. Water was obtained from a Milli-Q (Millipore, MA) water purification system.

2.2. Preparation of cyclosporin formulations

2.2.1. Cyclosporin EG formulations

EG formulations were prepared using a modified procedure based on the methods of Shively and Myers (Myers and Shively, 1992; Shively, 1993; Shively and Myers, 1993). Sucrose (5.45 g) and water (10 ml) were combined in a silanized 250 ml glass round bottom flask. Once the sucrose had dissolved with the aid of mild sonication, 1.55 g of olive oil (containing approximately 20 mg cyclosporin per gram) was added. The flask was then attached to a Buchi RE-111 (Switzerland) rotary evaporator, partially immersed in a water-bath maintained at 40°C and rotated at approximately 20 rpm. The vacuum was carefully applied to avoid bumping of the solution. This typically involved application of a low vacuum for 3–4 h (approximately 30 mm Hg) to remove the major proportion of the water, after which the vacuum was progressively increased over the subsequent 4 h period to a final level of approximately 1–2 mm Hg. Foaming typically occurred 1–2 h after application of the higher vacuum, after which the mixture was dried by maintaining the same vacuum, rotation speed and water bath conditions for a further 2 h period. The resulting solid foam was then stored under vacuum, over phosphorous pentoxide, at room temperature. The final dose consisted of 2.6 g of the emulsifiable glass formulation (containing 12.5 mg cyclosporin, 2.02 g of sucrose and 0.57 g of olive oil) filled into four size OOO hard gelatin capsules.

2.2.2. Cyclosporin surfactant-enhanced emulsifiable glass (SEEG) formulations

During development of the standard EG formulations, it was noted that a crude o/w emulsion formed within the round bottom flask as an apparent precursor to eventual foam formation. This observation prompted investigation into the potential for formulating systems using a pre-emulsification step where the oil was initially emulsified in the sucrose solution prior to rotary evaporation and glass formation. The choice of an acceptable oil and surfactant system was determined by their ability to form a stable primary emulsion, and to enhance the solubility of cyclosporin in the oil phase thereby leading to increased drug loading. Peanut oil and a 55:45 (v/v) blend of Span 80:Tween 80 was the system of choice.

In order to maximise the solubility of cyclosporin in the peanut oil, Span 80 was incorporated into the peanut oil prior to addition of cyclosporin. This addition enhanced the incorporation of cyclosporin up to 60 mg/g of peanut oil. The primary o/w emulsion was formed by adding 2.44 g of peanut oil (containing approximatley 60 mg/g cyclosporin and 0.27 g of Span 80) to 8.4 ml of distilled water containing 0.22 g of Tween 80. The system was emulsified by sonication using an ultrasonic disintegrator (MSE Soniprep 150, UK) fitted with an exponential microprobe operated at an output frequency of 23 KHz and an average probe amplitude of 50 μm. A uniform emulsion was formed after 4×20 s periods of sonication (during which the emulsion was ice cooled). Sucrose (4.34 g) was then added to the emulsion (2:1 ratio of sucrose to oil) and the mixture stirred using a magnetic stirrer until complete dissolution of the sucrose had occurred. The formulation was subjected to a further period of sonication (2×20 s) prior to being transferred to a 250 ml round bottom flask and rotary evaporated to dryness. Addition of the surfactants dramatically decreased the processing time. Only 0.5–1 h was required at the low vacuum to remove the major proportion of the water, after which foaming rapidly occurred (5–10 min) on application of the higher vacuum. The foam was dried and stored as described for the EG systems. SEEG formulation (1.4 g) (containing 25 mg of cyclosporin and approximately 408 mg peanut oil, 45 mg Tween 80, 55 mg Span 80 and 867 mg sucrose) was filled into two size OOO hard gelatine capsules for each dose.

2.2.3. Cyclosporin SEDDS formulation

The SEDDS formulations were prepared according to the method of Charman et al. (1992). Briefly, empty soft gelatin capsules (R.P. Scherer, Australia) were filled with the formulation using a syringe and sealed with hot gelatin. The optimized self-emulsifying formulation contained 30% (w/w) Tagat TO, 67.1% (w/w) Miglyol 812 and 2.9% (w/w) cyclosporin, and each capsule was filled to contain 25 mg of cyclosporin.

2.3. Characterization of cyclosporin formulations

2.3.1. EG and SEEG cyclosporin formulations

The microcrystallinity and water content of the EG and SEEG formulations were determined using differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA), respectively, employing Perkin Elmer DSC-7 and TGA-7 instruments (Perkin Elmer, CT). Samples to be analyzed for water content using the TGA apparatus were heated at 10°C/min under N_2 flowing at 30 ml/min. The DSC (calibrated daily with indium) was operated under a flowing high purity N_2 atmosphere. Samples were accurately weighed (2–6 mg) and compressed into 30 μl volatile aluminium sample pans prior to being sealed. An empty sample pan was used as a reference. Calorimetric data were then collected at a scan rate of 10°C/min under a N_2 flow rate of 20 ml/min through both the sample and reference compartments. The percent microcrystallinity ($\%X$) was calculated using the following equation based on the method of Peppas and Merrill (1976). Accordingly, $\%X = ((\Delta H_R + \Delta H'_m)/\Delta H_M) \times 100$ where ΔH_R is the sample enthalpy of recrystallization (exothermic); $\Delta H'_m$ is the sample enthalpy of melting (endothermic) and ΔH_M is the enthalpy of melting of pure sucrose.

The droplet size distribution of the reconstituted formulations was qualitatively assessed microscopically, and quantitatively assessed using a Coulter Multisizer II particle sizing apparatus (Coulter Electronics, USA). For microscopic evaluation, 2 ml of water was added to 300 mg of the EG or SEEG formulation, the sample shaken and an aliquot placed on a microscope slide. When using the Coulter Multisizer II system, 300 mg of the formulation was reconstituted with 2 ml of water, and a sample of the dispersed phase was added to the 100 ml volume cell containing normal saline.

The dispersion/rupture characteristics of the filled hard gelatin capsules containing the EG formulations were assessed using a thermostated (37°C) modified USP dissolution apparatus. The capsules were introduced into 400 ml of 0.1 N HCl in a 1 l glass vessel equipped with a standard dissolution paddle rotating at 100 rpm mounted immediately below the meniscus of the aqueous phase.

2.3.2. SEDDS cyclosporin formulation

The emulsification characteristics of the formulation were assessed using the dissolution procedure described for the EG and SEEG formulations. Briefly, a 0.3 ml aliquot of the SEDDS fill material was introduced into 400 ml of 0.1 N HCl in a 1 l glass vessel equipped with a standard dissolution paddle rotating as described above. Self-emulsification was allowed to proceed for 10 min after which a sample was withdrawn for particle sizing. The sample was diluted to contain approximately 1×10^5 particles/ml and analysed using a Coulter N4MD sizing apparatus (Coulter Electronics, USA).

2.4. Chromatography and analysis of cyclosporin

The cyclosporin content of the reconstituted IV, EG, SEEG and SEDDS formulations was determined using a Beckman System Gold HPLC (Beckman Instruments, CA) consisting of a Model 126 gradient pump and a Model 116 variable wavelength UV detector operated at 214 nm. Sample injection (20 μl) was via a WISP 717 autoinjector (Millipore, MA). Separation was achieved using an Aquapore RP300 (220 × 2.1 mm i.d) C8 column (Applied Biosystems, CA) operated at a flow rate of 0.4 ml/min and maintained at 45°C. A linear gradient utilizing 10% aqueous ACN (solvent A) and 80% aqueous ACN (solvent B) was used to elute cyclosporin when the percentage of solvent B was varied from 62 to 75% B over a 10 min period. The elution volume of cyclosporin was 2.4 ml. The assay was linear in

the concentration range 10–50 μg/ml ($r > 0.99$), and calibration standards were run at the beginning and end of each analysis. The content of cyclosporin in all formulations was quantified prior to utilization in the bioavailability study. For the reconstituted IV formulation (containing 0.5 mg cyclosporin per ml normal saline), an aliquot was diluted 1:20 with 53% ACN and subjected to HPLC analysis. For analysis of the EG and SEEG formulations, an accurately weighed sample (0.26 g) was placed in a 10 ml volumetric flask, 6 ml of ACN was added and the flask sonicated (Bransonic 220, CT) for between 5 and 10 min to disrupt the amorphous glass. The sample was then made to volume with ACN, an aliquot taken and centrifuged for 10 min to pellet the insoluble sucrose, and the supernatant diluted 1 in 5 with ACN prior to HPLC analysis. The absolute recovery of cyclosporin from the matrix was 90.4 ± 1.1% (Mean ± S.D., $n = 3$).

The cyclosporin content of the EG and SEEG was 4.8 and 18.8 mg/g of matrix, respectively. For the bioavailability study, empty size OOO hard gelatin capsules (Lilly, Australia) were individually filled with 650–700 mg of the formulation. The administered dose of cyclosporin was 12.5 mg for the EG formulation which corresponded to four capsules, and 25 mg of the SEEG formulation which corresponded to two capsules.

Analysis of the fill material used to prepare the SEDDS formulations involved placing an accurately weighed sample (50 mg) into a 10 ml volumetric flask and making up to volume with ACN. From this sample, a 1:5 dilution was then performed using 60% ACN prior to HPLC analysis. The absolute recovery of cyclosporin from the SEDDS matrix was 88.8 ± 1.4% (Mean ± S.D., $n = 3$).

The filled SEDDS and the Sandimmun® capsules were assayed by placing a capsule into a 200 ml volumetric flask, to which 50 ml of water was added and the solution was heated to 45–50°C until the gelatin shell was completely melted. The sample was made to volume with iso-propanol, an aliquot taken and diluted 1:5 with ACN prior to HPLC analysis. The recovery of cyclosporin was 95.4 ± 1.1% (Mean ± S.D., $n = 3$). The intravenous formulations were freshly prepared and assayed prior to each study day, and the stability of the EG, SEDDS and SEEG formulations was demonstrated prior to and during the course of the study.

2.5. Bioavailability evaluation of formulations

Two separate studies were conducted and each was approved by the local Institutional Animal Ethics Review Committee. The absolute bioavailability study was a randomized, single blind (analyst) four-way cross-over, and the relative bioavailability study was a randomized two-way cross-over both conducted in four male beagle dogs (age, 1.0–1.4 years; weight, 11–15 kg). The dogs were fasted for at least 12 h prior to each study day and water was available ad libitum. The washout period between each drug administration was 7 days.

Venous blood samples (2.5 ml) were taken, via an indwelling catheter in the cephalic vein, prior to medication (− 10 min) and at 0 (end of infusion), 5, 10, 15, 30 and 45 min and 1, 2, 3, 4, 6, 8, 10, 11, 12, 24 and 28 h post-medication after i.v. administration; and at − 10 min and 0.5, 1, 2, 3, 4, 6, 8, 9, 10, 24 and 28 h post-medication after oral administration. Blood samples were collected in sterile tubes containing 4.5 mg dipotassium EDTA.

Calculated pharmacokinetic parameters included the area under the blood concentration time profile from − 10 min (start of infusion) to time infinity ($AUC^{-10\rightarrow\infty}$) after i.v. administration, and from time zero to infinity ($AUC^{0\rightarrow\infty}$) for the oral administrations as well as the maximum blood concentration (C_{max}), the time taken to reach C_{max} (t_{max}), and the terminal elimination half-life ($t_{1/2}$). The AUC data were calculated using the linear trapezoidal rule to the last measured blood concentration and adding to that the extrapolated area calculated by dividing the last measured blood concentration by the terminal elimination rate constant. The absolute bioavailability of cyclosporin from the different formulations was calculated as the ratio of the dose normalized AUC values after oral and i.v. administration according to standard procedures (Gibaldi and Perrier, 1982).

2.5.1. Absolute bioavailability study of EG, SEDDS and Sandimmun® formulations

Each dog randomly received, in cross-over fashion, either (i) Sandimmun® i.v. formulation (10 ml of a 0.5 mg/ml solution infused over a 10 min period), (ii) a 25 mg Sandimmun® capsule, (iii) a SEDDS formulation containing 25 mg cyclosporin, or (iv) four size OOO hard gelatin capsules containing the EG formulation (12.5 mg dose of cyclosporin).

2.5.2. Relative oral bioavailability of SEEG and Sandimmun® formulations

Each dog randomly received, in cross-over fashion, either a 25 mg Sandimmun® capsule, or two size OOO hard gelatin capsules containing the SEEG formulation (25 mg dose of cyclosporin). The oral bioavailability of cyclosporin from the SEEG (test) formulation was calculated relative to the commercial (reference) formulation according to standard procedures (Gibaldi and Perrier, 1982).

2.6. Quantitation of cyclosporin in blood samples

Whole blood concentrations of cyclosporin were determined using a monoclonal fluorescence polarization immunoassay procedure (TDx system, Abbott Diagnostics) operated according to the manufacturer's written specifications. The assay involved pretreatment (solubilization and precipitation) of 150 μl whole blood sample, centrifugation, after which the supernatant was loaded into sample cartridges for TDx analysis. Control and calibrator (whole blood) samples were treated in a similar manner. The assay was linear over the concentration range 10–1500 ng/ml, and the CV was less than 5% at all concentrations. In the concentration range 50–1500 ng/ml, a 50 μl sample volume is the default volume required for analysis by the TDx apparatus. The sensitivity of the assay was extended to 10 ng/ml by programming a 200 μl sample volume for analysis when the nominal concentration was below 50 ng/ml. The CV for spiked blood concentrations in this range remained below 5%.

2.7. Statistical evaluations

The statistical significance of differences in C_{max} and AUC data for the oral formulations were evaluated using a one way, repeated measures analysis of variance (ANOVA). T_{max} values were compared using the non-parametric Kruskal-Wallis one way ANOVA on ranks test, and the test formulations subsequently compared with the reference using Dunns test for multiple comparisons.

3. Results

3.1. Characterization of cyclosporin formulations

The cyclosporin EG formulation was a dry foam-like solid matrix which could easily be broken down with a spatula. Thermograms of the EG formulations exhibited glass transitions at approximately 45°C, a recrystallization peak onset at approximately 70°C and a crystalline melting peak onset at 170°C. The calculated microcrystallinity was 35%, and the final water content was typically 2.5% (w/w). Upon reconstitution of many different solid EG samples in either water, normal saline or 0.1 N HCl, some oil was present as a dispersed phase although there were always significant quantities of residual oil which floated on the surface of the reconstitution liquid. The particle size distribution of the oil which dispersed upon reconstitution was broad (Table 1).

In contrast, the SEEG systems were found to rapidly and completely emulsify (no residual oil) upon reconstitution to produce a uniform emulsion with a mean particle size of approximately 1 μm. Although the physical appearance of the SEEG formulation was similar to the EG formulations, DSC and TGA analysis indicated notable differences as the SEEG formulation was entirely microcrystalline and essentially dry (Table 1).

The SEDDS formulation was included in the absolute bioavailability study as a positive control as it produced an extremely fine emulsion on reconstitution, and the pertinent data are presented in Table 1. Under the in vitro dissolution procedures employed, the commercial cyclosporin formulation (Sandimmun®) did not self emulsify.

Table 1
Comparison of the relevant formulation and physicochemical characteristics of the emulsified glass (EG), liquid self-emulsifying drug delivery system (SEDDS), and surfactant enhanced emulsified glass (SEEG) formulation

Parameter	Formulation		
	EG	SEDDS	SEEG
Drug loading (mg cyclosporin per gram of fill)	4.8	30.0	18.8
Dose (mg) administered	12.5	25	25
Number of capsules per dose	4	1	2
Microcrystallinity (%)	35	N/A[a]	100
Water content (%)	2.5	N/A	0.02
Particle size (μm) of dispersed phase formed on reconstitution	7.3 ± 5.5[b]	0.20 ± 0.07	1.15 ± 0.36

[a] Not applicable.
[b] Incomplete emulsification, significant quantities of residual oil present.

3.2. Absolute bioavailability of EG, SEDDS and Sandimmun® formulations

The oral formulations (25 mg Sandimmun®, 25 mg SEDDS and 12.5 mg EG) were well tolerated and there were no observable side effects. However, towards the later stages of the i.v. infusion each dog developed, to varying degrees, a self-limiting mild analphylactoid-type reaction (facial flushing, itchiness, redness of the ears). This response is consistent with human experience where histamine release from mast cells is induced by the polyethoxylated castor oil derivative (Cremophor EL) present in the formulation. The mean cyclosporin blood concentration versus time profile after i.v. administration is presented in Fig. 1. The calculated (Mean $\pm$ S.D., $n = 4$) terminal elimination rate constant was 0.033 ± 0.004 h^{-1}, the clearance was 166.5 ± 38.4 ml/h/kg and the volume of distribution during the terminal elimination phase was 5.19 ± 0.97 l/kg.

The mean cyclosporin blood concentration versus time profiles after administration of the oral formulations are presented in Fig. 2. The C_{max}, T_{max}, AUC and bioavailability data for the these formulations are presented in Table 2. The cyclosporin blood concentration time profiles after administration of the 25 mg Sandimmun® and SEDDS formulations were not statistically different ($\alpha = 0.05$) with respect to C_{max} and absolute bioavailability. However, the maximum blood concentration of cyclosporin occurred earlier after administration of Sandimmun® formulation relative to the SEDDS formulation ($p < 0.05$). After normalization of the data from the EG formulation (to an equivalent 25 mg dose) the systemic blood concentrations of cyclosporin were comparable with that obtained after administration of either Sandimmun® or the SEDDS formulation. However, there was greater variability after administration of the EG formulation as demonstrated by the large standard deviations associated with the mean values for absolute bioavailability and C_{max}.

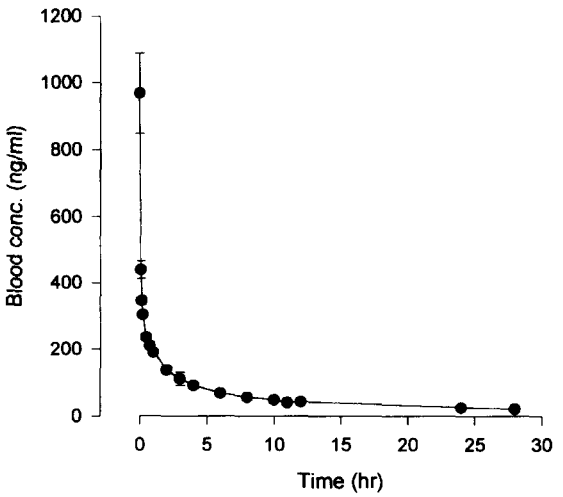

Fig. 1. Mean ($\pm$ S.E., $n = 4$) blood cyclosporin concentration vs time profiles after i.v. administration to fasted beagle dogs (10 ml of a 0.5 mg/ml solution infused over a 10 min period).

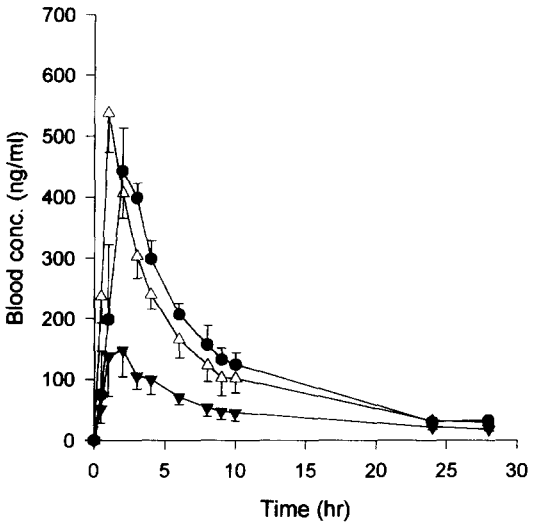

Fig. 2. Mean (± S.E., $n = 4$) blood cyclosporin concentration vs time profiles after administration of 25 mg Sandimmun® oral formulation (△ – △), 25 mg SEDDS formulation (● – ●), and the 12.5 mg EG formulation (▼ – ▼) to fasted beagle dogs.

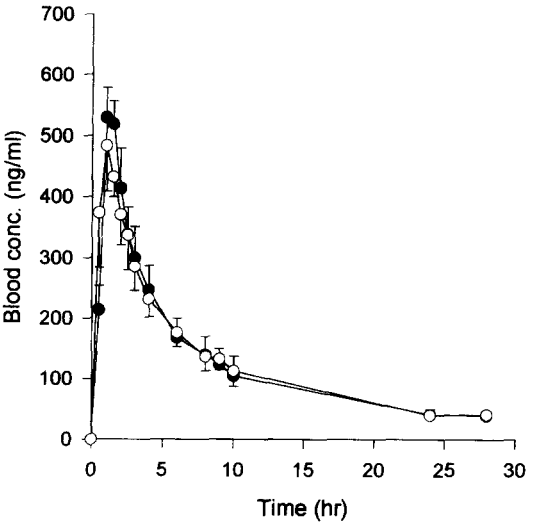

Fig. 3. Mean (± S.E., $n = 4$) blood cyclosporin concentration vs time profiles after administration of 25 mg Sandimmun® oral formulation (● – ●) and the 25 mg SEEG formulation (○ – ○) to fasted beagle dogs.

3.3. Relative bioavailability of 25 mg SEEG and Sandimmun® formulations

This study examined the bioavailability of the SEEG formulation relative to oral Sandimmun® (comparator formulation between the two studies). Both formulations were well tolerated. The mean cyclosporin blood concentration versus time profiles after administration of Sandimmun® and the SEEG formulation are presented in Fig. 3, and the pertinent pharmacokinetic parameters are described in Table 3. Within the limitations of a small study group ($n = 4$), there were no statistically significant differences ($\alpha = 0.05$) in C_{max}, T_{max} or AUC between Sandimmun® and the SEEG formulation. The bioavailability of Sandimmun® was highly consistent across the two studies as judged from the similarity in the AUC and C_{max} data.

Table 2
Mean (± S.D., $n = 4$) pharmacokinetic parameters, and absolute and relative bioavailability of cyclosporin, after the randomized cross-over administration of Sandimmun®, the emulsifiable glass (EG) formulation, and a liquid self emulsifying formulation (SEDDS) to fasted beagle dogs

Parameter	Formulation		
	Sandimmun®	EG	SEDDS
Dose (mg)	25	12.5	25
C_{max} (ng/ml)	537.5 ± 127.7	198.2 ± 108.2	494.8 ± 60.4
T_{max} (h)	1.0 ± 0.0	1.8 ± 1.0	2.3 ± 0.5
$AUC^{0\rightarrow\infty}$ (ng·h/ml)	4168 ± 1304	1704 ± 625	4464 ± 855
Absolute BA (%)	33.6 ± 2.5	27.9 ± 8.4	36.5 ± 2.5
Relative BA (%)	100	86.7 ± 36.8	112.4 ± 29.0

Table 3
Mean (± S.D., $n = 4$) pharmacokinetic parameters and relative bioavailability of cyclosporin, after the randomized cross-over administration of Sandimmun® and the surfactant enhanced emulsifiable glass (SEEG) formulation to fasted beagle dogs

Parameter	Formulation	
	Sandimmun®	SEEG
Dose (mg)	25	25
C_{max} (ng/ml)	554.3 ± 65.1	547.7 ± 88.6
T_{max} (h)	1.1 ± 0.3	1.1 ± 0.6
$AUC^{0\rightarrow\infty}$ (ng.h/ml)	4569 ± 1529	4532 ± 1288
Relative BA	100	104.2 ± 33.4

4. Discussion

4.1. Emulsifiable glasses prepared from sucrose:oil mixtures

Emulsifiable glasses are a potential approach for the convenient co-administration of lipids and lipophilic drugs (Myers and Shively, 1992; Shively and Myers, 1993). The current studies were undertaken to characterise and evaluate the EG technology, and to assess the bioavailability of a model lipophilic compound (cyclosporin) when formulated as an emulsifiable glass. The EG formulations contained a sucrose:oil ratio of 3.5:1 and were prepared employing the procedures reported by Myers and Shively (1992). The thermal, microcrystallinity and physicochemical characteristics of the cyclosporin EG formulations were broadly similar to those described by Myers and Shively (1992) for 'drug free' formulations. Following reconstitution of the cyclosporin EG, the mean particle size of the dispersed phase was similar to that reported by Myers and Shively (1992) for drug free EG formulations. However, significant quantities of non-emulsified oil were always present after reconstitution of the cyclosporin EG formulations.

Myers and Shively (1992) reported the presence of residual oil upon reconstitution of EG formulations prepared using sucrose:oil ratios less than 2.5:1, but stated that the problem could be overcome by increasing the proportion of sucrose in the formulation above 2.5:1. The EG formulation used in this study was formulated using 3.5:1 sucrose:oil and was expected to efficiently self-emulsify. It is possible that the presence of cyclosporin reduced the emulsification of the EG, however, residual oil was still evident when drug free EG formulations of the same sucrose:oil compositions were prepared and reconstituted. Numerous approaches, which have included different sucrose:oil ratios, processing temperatures and times, and vacuum levels have been evaluated in an attempt to prepare sucrose:oil EG formulations which efficiently emulsified. Unfortunately, all of these attempts were unsuccessful.

Notwithstanding the problem of residual oil, the absolute bioavailability of cyclosporin from the EG (12.5 mg) was determined in a cross-over study conducted in beagle dogs. The comparator oral treatments were the commercially available Sandimmun® capsule (25 mg) and a specifically designed, highly efficient liquid 25 mg self-emulsifying lipid formulation (Charman et al., 1992). Cyclosporin was administered i.v. to enable determination of the absolute bioavailability of the oral formulations. Clearance, volume of distribution and elimination half-life of cyclosporin after i.v. administration were similar to published data (Ptachcinski et al., 1987; Gupta et al., 1990). The data in Table 2 and Fig. 2 indicated that the absolute oral bioavailability of cyclosporin from the three different oral formulations was similar, although there was marked variability in the AUC values after administration of the EG formulation. The variability may reflect the incomplete emulsification observed in vitro, or it may simply be a function of having to administer four separate capsules to provide the 12.5 mg dose. In contrast, the plasma profiles and related AUC data were highly reproducible after administration of either the SEDDS or Sandimmun® formulations. The in vivo performance of the SEDDS formulation was consistent with previous work (Charman et al., 1992).

4.2. Emulsifiable glasses prepared from surfactant enhanced sucrose:oil mixtures

The incomplete emulsification, limited drug incorporation and variable absorption of cyclosporin after administration of the EG formulation prompted an investigation into the potential for enhancing the performance of the EG by addition of a limited amount of an appropriate surfactant. In contrast to liquid SEDDS formulations, which require 30–40% surfactant, the surfactant enhanced emulsifiable glass (SEEG) formulations developed in this study required less than 10% surfactant to produce reproducible emulsified systems after reconstitution. Furthermore, the surfactant employed in the SEEG formulations was GRAS status unlike many of the non-ionic surfactants used with SEDDS formulations (eg. Tagat TO).

In contrast to the EG formulation, the SEEG formulation completely dispersed after reconstitution to yield an emulsion with a narrow particle size distribution (Table 1). Furthermore, addition of a surfactant to the oil phase increased the lipid solubility of the cyclosporin, and sufficiently enhanced the emulsification properties to enable reduction of the sucrose:oil ratio to 2:1. The combination of these two factors increased the drug loading of the SEEG formulation up to four-fold relative to the EG formulation.

Thermal analysis of the SEEG formulation indicated the absence of amorphous material as there was no glass transition or recrystallization peak present in the thermogram. Therefore, the sample was assumed to be 100% microcrystalline. A potential explanation for the highly microcrystalline character of the SEEG is that the surfactant may have decreased the surface energy of the oil/glass or the glass/crystal interface thereby facilitating the nucleation process. Although the SEEG was of similar physical appearance to the EG, the SEEG solids were not strictly glasses as they were no longer amorphous. Consequently, 'glass-like' character was not a prerequisite for efficient emulsification in the surfactant enhanced systems. Indeed, Shively and Myers (1993) indicated that the degree of microcrystallinity was independent of the efficiency of self emulsification for their EG systems.

The in vivo performance of the SEEG formulation was similar to Sandimmun® when compared in a two way cross-over study conducted in fasted beagle dogs (Table 3 and Fig. 3). Comparing across studies, the performance of Sandimmun® was highly reproducible in terms of C_{max}, T_{max} and AUC. Each of these parameters was also less variable after administration of the SEEG formulation compared with the EG formulation.

The rank order droplet size of the 'emulsion/dispersed lipid' formed after dissolution analysis of the SEDDS, EG, SEEG and Sandimmun® formulations was SEDDS (uniform emulsion of approximately 0.2 μm) < SEEG (uniform emulsion of approximately 1 μm) < EG (containing non-dispersed lipid and dispersed lipid of approximately 5 μm) < Sandimmun® (the contents of which did not disperse very well under the conditions employed). Within the limitations associated with cross-study comparisons, the blood concentration versus time profiles of cyclosporin from the different formulations indicated that the absorption of cyclosporin was relatively insensitive to the degree of dispersion (based on in vitro dissolution results).

The stomach plays an important role in the initial gastric processing of lipids as it facilitates formation of a crude emulsion through minor lipolysis and the shear associated with gastric emptying. Consequently, the similar extents of cyclosporin absorption from the formulations which produced different in vitro dispersion profiles may reflect 'gastric processing' of the lipid-based formulations to an extent where the physical states of the drug and associated lipid which emptied from the stomach were similar.

In summary, EG formulations based on the methods of Myers and Shively (1992) have been applied to cyclosporin and evaluated both in vitro and in vivo. The limited drug loading capacity and incomplete emulsification characteristics of the EG formulation were improved by developing a surfactant enhanced system (SEEG). Although the drug loading capacity of these systems is still relatively low, for potent, lipophilic compounds, solid SEEG formulations may provide advantages in administration and chemical stability over traditional formulation alternatives such as emulsions and liquid fill softgels.

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