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Improved oral bioavailability of cyclosporin A in male Wistar rats
Comparison of a Solutol HS 15 containing self-dispersing formulation and a microsuspension

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Abstract

Oral bioavailability of the highly lipophilic and poorly water-soluble immunosuppressive agent cyclosporin A (CyA) in two different formulations was investigated in male Wistar rats. An aqueous microsuspension and a self-dispersing formulation composed of the surface-active ingredients Solutol HS 15:Labrafil M2125CS:oleic acid = 7:2:1 (v/v/v) were administered to the animals at a dose level of 20 mg/kg. In order to calculate the absolute oral bioavailability, CyA was additionally administered intravenously at 10 mg/kg as microsuspension. It was found that the oral bioavailability of CyA in the Solutol HS 15-based formulation was twofold higher as compared to the microsuspension (69.9 ± 2.8 vs. 35.7 ± 3.3%, $P = 0.001$). By contrast, the time to reach maximum plasma concentration (t_{max}) and the terminal half-life ($t_{1/2}$) did not differ significantly with the different formulations (t_{max}: 7.0 ± 1.0 vs. 6.3 ± 1.7 h; $t_{1/2}$: 20.5 ± 2.9 vs. 16.7 ± 4.7 h). In vitro solubility experiments demonstrated a marked increase in the aqueous solubility of CyA in the presence of the self-dispersing formulation as compared to the micronized powder alone (solubility after 120 min at 37 °C: 136 vs. 23.2 μg/ml in human gastric juice; 133 vs. 10.8 μg/ml in simulated intestinal juice). Most likely, the enhanced systemic exposure of CyA in the self-dispersing formulation was caused by improved solubility of CyA in the gastrointestinal fluids in the presence of the surface-active ingredients. Additional factors that may have contributed to increased oral bioavailability are inhibition of metabolism and/or transport processes as well as permeability enhancement by the co-administered excipients.

Keywords: Cyclosporin A; Solutol HS 15; Self-dispersing formulation; Microsuspension; Oral bioavailability

1. Introduction

The cyclic polypeptide cyclosporin A (CyA) is a potent immunosuppressive agent used to inhibit organ rejection in transplant patients and for the treatment of autoimmune disorders (Fahr, 1993).

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The oral bioavailability of CyA, however, is highly variable (Ptachcinski et al., 1986) and generally low with an overall mean of 29% (Kahan, 1985). A number of different reasons have been suggested for the low and erratic systemic exposure upon oral administration. For example, CyA is prone to extensive gut wall (Tjia et al., 1991) and hepatic metabolism (Ptachcinski et al., 1986), with cytochrome P450 3A being the major enzyme involved in this process (Kelly et al., 1999). Additionally, the efflux transporter p-gp seems to play a major role in the inter-individual variability in the oral bioavailability of CyA (Saeki et al., 1993; Lown et al., 1997). The proposed existence of an absorption window in the small intestine may also complicate quantitative absorption of the compound (Drewe et al., 1992). This is particularly important since CyA is highly lipophilic and poorly water-soluble (Ismailos et al., 1991). Thus, its oral administration in particulate form (e.g. as tablet or capsule formulation) would not guarantee quantitative dissolution in the upper part of the gastrointestinal tract.

A variety of formulation principles such as mixed micelles (Takada et al., 1985), liposomes (Ueda et al., 1981), lipids (Ueda et al., 1983), surfactants (Chang et al., 1996), or emulsions (Ritschel et al., 1990) have been investigated in order to improve the unfavorable absorption characteristics of CyA. The first marketed oral formulation Sandimmune was based on olive oil, Labrafil (peglicol-5 oleate), and alcohol (Johnston et al., 1986). Upon dilution with aqueous phase, this formulation forms a crude O/W (oil-in-water) emulsion. For the subsequent formation of mixed micelles promoting the absorption of CyA, the presence of bile and pancreatin is mandatory. Therefore, the Sandimmune formulation results in a comparatively low oral bioavailability along with a high inter-subject variability (Metha et al., 1984; Friman and Bäckman, 1996). These disadvantages of Sandimmune can be overcome, in parts, with the follow-up CyA formulation Sandimmune Neoral (Kovarik et al., 1994; Taesch and Niese, 1994; Friman and Bäckman, 1996). In Sandimmune Neoral CyA is dispersed in a mixture of the hydrophilic co-solvent propylene glycol, mono-, di- and triglycerides from corn oil, polyoxyl-40 hydrogenated castor oil as surfactant, and the antioxidant DL-tocopherol (Levy and Grant, 1994). Upon dilution with aqueous phase, this self-emulsifying formulation forms a fine and homogenous microemulsion with droplet sizes of below 100 nm, thereby preventing precipitation of incorporated CyA. In renal transplant recipients, the prehepatic intestinal metabolism of CyA was not altered in the presence of the Sandimmune Neoral dosing vehicle as compared to Sandimmune (Vernillet et al., 1994). Thus, it is unlikely that improved oral bioavailability and/or reduced intra- and inter-individual variability result from inhibition of CyA metabolism due to ingredients of the Sandimmune Neoral formulation.

Nowadays, in industrial drug discovery and development, an increasing number of compounds are characterized as being sparingly water-soluble and highly lipophilic. Since these unfavorable physicochemical features may result in low and highly variable oral bioavailability as described for CyA, suitable formulation principles need to be explored intensively (Bittner and Mountfield, 2002). For example, the surface-active formulation ingredient Solutol HS 15, the main component of which is the polyethylene glycol 660 ester of 12-hydroxy stearic acid, shows the potential to increase the solubility of a number of sparingly water-soluble and highly lipophilic compounds (F. Hoffmann-La Roche, data on file). Solutol HS 15 has been approved in a parenteral Phytonadion formulation for human use on the Canadian market. Studies on its suitability to improve the oral bioavailability of co-administered compounds, however, are still missing in the literature.

The aim of the current investigation was, therefore, to develop a Solutol HS 15-based dosing vehicle capable of enhancing the oral bioavailability of the model compound CyA. The formulation was characterized for its potential to prevent precipitation of co-formulated CyA upon dilution with gastrointestinal fluids in vitro as well as to enhance the oral bioavailability of CyA in male Wistar rats. An aqueous microsuspension of CyA, which can be considered to be composed of 'non-interfering' ingredients, was used as reference formulation.

2. Materials and methods

2.1. Materials

CyA was purchased from Fluka (Buchs, Switzerland). The solubility of CyA was investigated in Solutol HS 15 (BASF, Ludwigshafen, Germany), Labrafil M2125CS (Gattefossé, Saint Priest, France), oleic acid (Fluka), sesame oil (Fluka), polyethylene glycol 200, 300 and 400 (Fluka), propylene glycol (Fluka), *N*,*N*-dimethylacetamide (Fluka), glycofurol 75 (Roche, Basel, Switzerland), *N*-methylpyrrolidone (Fluka), soybean oil (Fluka), glycerol (Fluka), Labrafil WL2609 (Gattefossé), Labrafac CC (Gattefossé), Labrafac CM 10 (Gattefossé), Labrasol (Gattefossé), Tween 80 (Fluka), and Tween 20 (Fluka). All other materials used were of analytical grade or are described separately in the methods section.

2.2. Formulations for in vivo experiments

For in vivo experiments in male Wistar rats, CyA was formulated either as microsuspension or as self-dispersing formulation (formulation 1). Gershanik and Benita (2000) have reviewed the characteristics of self-dispersing formulations in detail. The dosing concentration of CyA in both vehicles was 6 mg/ml. CyA was obtained as milled powder with particle sizes ranging from 1 to 5 μm. In order to further reduce particle size and to homogenize particle size distribution, a microsuspension was prepared. Circa 500 μl of an aqueous vehicle containing gelatin (7.5%, B. Braun Medical AG, St. Gallen, Switzerland) and sodium chloride (0.62%, Fluka) were added to 36.0 mg of the compound. After addition of 1 g glass beads (MK 26X, ∅ 0.25–0.5 mm, Willy A. Bachofen AG, Basel, Switzerland), the mixture was milled in a Retsch shaker (MM200, Schieritz and Harnstein AG, Arlesheim, Switzerland) for 3 h. After removal of the glass beads by filtration, the suspension was diluted to 6 ml with the aqueous vehicle. As shown light microscopically, particle size distribution of the microsuspension was very homogenous ranging from 0.5 to 1 μm. In order to prepare the self-dispersing formulation, CyA was dissolved in the Solutol HS 15:Labrafil M2125CS:oleic acid = 7:2:1 mixture (liquefied by gentle heating to 37 °C) at 100 mg/ml. Three hundred microliters of this solution was pipetted into 4700 μl of water. The mixture was stirred for 5 min at room temperature and a slightly turbid emulsion was formed. For intravenous administration, CyA was formulated as microsuspension at 5 mg/ml as described above.

2.3. Solubility of CyA in human gastric and simulated intestinal juice

In order to determine the solubility of CyA in the presence and in the absence of formulations 1 (Solutol HS 15:Labrafil M2125CS:oleic acid = 7:2:1) and 2 (Solutol HS 15:Labrafil M2125CS:oleic acid:sesame oil = 4.9:1.4:0.7:3), either 0.136 mg of micronized CyA or 1.36 μl of the respective surfactant-based formulations (concentration: 100 mg/ml) were added to 1 ml of human gastric juice (Kantonsspital, Basel, Switzerland) or simulated intestinal juice. The samples were stirred for 120 min at 37 °C. To determine the concentration of dissolved CyA, after 0.5, 30, 60, and 120 min the samples were centrifuged at 5000 rpm for 5 min. The clear supernatant was analyzed by High Performance Liquid Chromatography (HPLC). All samples were assayed in triplicate.

The simulated intestinal juice was composed as follows: 6.8 g monobasic potassium phosphate (Fluka), 77 ml 0.2 N sodium hydroxide (Fluka), 10.0 g pancreatin (Fluka), and 750 ml demineralized water. The pH of the solution was adjusted to 6.8 with 0.2 N sodium hydroxide.

2.4. HPLC method for CyA

The HPLC system consisted of the following modular components: pump (HP 1100 quaternary pump G 1311A, Agilent Technologies GmbH, Waldbronn, Germany), detector (HP 1100 diode array detector G 1315A, Agilent Technologies GmbH, Waldbronn, Germany), column (μ-Bondapak C_{18}, 3.9 × 300 mm, Waters, Ireland). The mobile phase consisted of 20% of acetonitrile:20 mM ammonium acetate pH 4 (5:95), and 80% of acetonitrile: 20 mM ammonium acetate pH 4 (95:5). The flow rate was 1.5 ml/min, the run

time was 10 min, and the injection volume was 20 μl. Detection was carried out at 225 nm. CyA standards from 6.25 to 200 μg/ml were prepared in methanol. CyA was stable in formulation 1 at 100 mg/ml at room temperature, at 25 and 37 °C over a period of 24 h.

2.5. In vivo experiments

Male Wistar rats (BRL, Füllinsdorf, Switzerland) weighing circa 250 g were used for in vivo studies. The animals were housed in standard cages and maintained under a 12 h light/dark cycle with access to standard laboratory chow and water ad libitum. For kinetic studies, all rats had an indwelling cannula (silicone rubber/polyethylene) implanted in the right jugular vein for blood sampling. Surgery was performed under 10 mg/kg xylazin (Ketasol-100, Graeub, Bern, Switzerland) and 90 mg/kg ketamin (Rampun®, Bayer, Lyssach, Switzerland) anesthesia 2 days before the experiment. During the study, all animals were housed individually in plastic metabolism cages and were unrestrained throughout the experimental time period. For oral experiments rats received a single dose of CyA (20 mg/kg), formulated either as microsuspension ($n = 3$) or in formulation 1 ($n = 3$). For intravenous experiments the animals received a single dose of CyA (10 mg/kg) as microsuspension. Blood samples (0.3 ml) were collected from the jugular vein at defined intervals. Collection tubes contained ethylendiamin-tetraacetic acid (EDTA, Fluka) and sodium fluoride (Fluka) as anticoagulant and stabilizer, respectively. Plasma samples were obtained by immediate centrifugation of blood samples. All samples were kept frozen at −20 °C until assayed by LC/MS–MS (see Section 2.7).

2.6. Data analysis

Maximum plasma concentration (c_{max}) and time to reach c_{max} (t_{max}) were recorded directly from experimental observations. The area under the plasma concentration–time profile (AUC) was calculated using the log–linear trapezoidal method in WinNonlin (Version 1.5, Scientific Consulting Inc., USA), as was the elimination half-life, which was estimated from the slope of the terminal phase of the log plasma concentration–time points fitted by the method of least-squares. Additional calculated parameters were systemic plasma clearance (Cl) and the volume of distribution (V_d). P values were calculated with a two-sample t-test in excel assuming equal variances. P values of below 0.03 were considered as being statistically significant.

2.7. LC/MS–MS

A specific and selective LC/MS–MS method was used to quantify CyA in plasma samples. To analyze the plasma samples, a 50 μl sample aliquot of plasma was diluted with two volumes of methanol and samples were centrifuged to precipitate proteins. The supernatant (100 μl) was then again diluted with one volume of water and 30 μl were injected into a 20 μl loop of a reversed phase HPLC system. A two-component mobile phase, pumped at 0.2 ml/min, contained the following solvents: solvent A (20 mM ammonium acetate pH 4 in water) and solvent B (acetonitrile). The column (Symmetry C8, 50 × 2.1 mm ID, 5 μm particle size, Waters, Milford, MA, USA) was heated to 70 °C. An initial isocratic period of 0.5 min was followed by a gradient from 40 to 95% B within 0.5 min, again followed by an isocratic phase of 2 min. Detection was performed with an API3000 mass spectrometer (Sciex, Foster City, Canada) equipped with a Turbo-Ionspray source. After passing the interface (350 °C, 7 l N_2/min, 5.5 kV), the eluent was monitored with the selected reaction monitoring mode (SRM) transition 1202.6 → 199.2 (CE 95 eV) to quantify CyA. The overall run-time of the method was 4 min with CyA eluting after 2.1 min. Quantitative and qualitative analysis were performed using the Analyst 1.1 software (Sciex, Foster City, Canada). Concentrations of study and quality control samples were calculated from calibration curves established by linear least-squares regression analysis. CyA demonstrated stability in rat plasma after storage at room temperature for 24 h.

3. Results

The immunosuppressive CyA is an interesting model compound for developing oil- and surfactant-based dosing vehicles suitable to orally administer compounds with similar physicochemical characteristics, thereby eventually increasing their oral bioavailability. The aqueous solubility of CyA is with about 10 μg/ml comparatively low, but due to its lipophilic nature (log $P = 2.92$; data on file, F. Hoffmann-La Roche) its solubility especially in oily formulation ingredients is usually high. As shown in Table 1, the solubility of CyA in different organic water-miscible co-solvents, in long-chain triglycerides, as well as in a number of surface-active formulation ingredients was greater than 100 mg/ml. Based on these preliminary solubility data, two CyA containing formulations consisting of either Solutol HS 15:Labrafil M2125CS:oleic acid = 7:2:1 (formulation 1) or Solutol HS 15:Labrafil M2125CS:oleic acid:sesame oil = 4.9:1.4:0.7:3 (formulation 2) were prepared at a concentration of 100 mg CyA per ml each. The choice of formulation ingredients was based on the prerequisite that Solutol HS 15 had to be the major formulation component. Mixtures with CyA, Solutol HS 15, and the different excipients were prepared and diluted with water. Dilutions were viewed in the microscope and the most homogenous mixtures were selected for further investigations.

Table 1
Solubility of CyA in various formulation ingredients

Formulation ingredient	Solubility (mg/ml)	Description
Polyethylene glycol 400	> 100	Water-miscible organic co-solvent
Polyethylene glycol 300	> 100	Water-miscible organic co-solvent
Polyethylene glycol 200	> 100	Water-miscible organic co-solvent
Propylene glycol	> 100	Water-miscible organic co-solvent
N,*N*-Dimethylacetamide	> 100	Water-miscible organic co-solvent
Glycofurol 75	> 100	Water-miscible organic co-solvent
N-Methylpyrrolidone	> 100	Water-miscible organic co-solvent
Sesame oil	> 100	Long-chain triglyceride
Soybean oil	$100 > X > 50$	Long-chain triglyceride
Glycerol	< 12.5	
Labrafil WL 2609	> 100	Unsaturated polyglycolized glyceride (C16–C20)
Labrafil M 2125	> 100	Unsaturated polyglycolized glyceride (C16–C20)
Labrafac CC	> 100	Medium-chain triglyceride
Labrafac CM 10	> 100	Polyglycolized glyceride (C8–C10)
Labrasol	> 100	Saturated polyglycolized glyceride (C8–C10)
Oleic acid	> 100	Long-chain fatty acid
Tween 80	> 100	Polyoxyethylene sorbitan monooleate
Tween 20	> 100	Polyoxyethylene sorbitan monolaureate
Solutol HS 15	> 100	Polyethylene glycol 660 12-hydroxy-stearate

In order to rank both formulations according to their ability to prevent precipitation of incorporated CyA in gastrointestinal fluids, a solubility study in human gastric juice and in simulated intestinal juice was performed. CyA was introduced into the media that were preheated to 37 °C either as powder or as solution in formulation 1 or 2, respectively. In the following, the expression 'dissolved CyA' refers to both CyA molecules free in solution and CyA molecules dissolved in micellar structures formed together with the surface-active excipients and amphiphilic ingredients in the physiological fluids. As shown in Table 2, after 120 min of incubation at 136 μg/ml, the concentration of dissolved CyA measured in the absence of the formulation ingredients was 23.2 μg/ml in human gastric juice and 10.8 μg/ml in simulated intestinal juice. This solubility advantage in human gastric juice was significant already after 30 min of incubation ($P < 0.03$). As it has been shown that the solubility of CyA is not affected by the pH of the aqueous environment (Ismailos et al., 1991), the different composition of human gastric juice and simulated intestinal juice seems to be responsible for the observed solubility difference.

In the presence of formulation 1, CyA was quantitatively dissolved in human gastric juice and the concentration did remain constant (136

Table 2
Solubility of CyA in simulated intestinal juice and in human gastric juice in the presence and in the absence of formulations 1 and 2

	CyA dissolved at 0.5 min (μg/ml)	CyA dissolved at 30 min (μg/ml)	CyA dissolved at 60 min (μg/ml)	CyA dissolved at 120 min (μg/ml)
Powder+human gastric juice	3.99±0.09	10.77±0.05	19.35±0.37	23.19±0.17
Powder+simulated intestinal juice	3.89±0.37	6.56±0.01	8.23±0.43	10.81±0.39
Formulation 1+human gastric juice	135.77±0.15	136.05±0.11	135.95±0.17	136.14±0.16
Formulation 1+simulated intestinal juice	135.67±0.14	133.54±0.05	131.86±0.16	132.54±0.21
Formulation 2+human gastric juice	122.80±0.16	122.92±0.03	122.91±0.16	121.78±0.11
Formulation 2+simulated intestinal juice	96.71±0.07	88.19±0.05	72.90±0.16	56.58±0.21

The samples were incubated at 37 °C over a period of 120 min ($n=3$), centrifuged after 0.5, 30, 60, and 120 min and the clear supernatant was assayed by HPLC, mean±SEM.

μg/ml) from t_0 up to t_{120}. In simulated intestinal juice in the presence of formulation 1, all of the CyA was dissolved as well. There was only a marginal decrease in the concentration of dissolved CyA over time (2.2% after 120 min of incubation). In the presence of formulation 2, the solubility of CyA in human gastric juice was 10% lower as compared to formulation 1. No precipitation was observed up to 120 min of incubation. By contrast, in simulated intestinal juice in the presence of formulation 2, the solubility of CyA at t_0 was comparatively low (96.7 μg/ml) and further decreased by about 41.5% at t_{120}. Thus, in the dosing vehicle containing sesame oil (formulation 2), CyA precipitation in the presence of aqueous phase could not be prevented as efficiently as in formulation 1. It is known from the literature that edible oils that do not possess intrinsic amphiphilic properties (such as sesame oil) are less suitable than surface-active agents for the design of self-dispersing systems. The ingredients used in formulation 1 are all amphiphilic by nature. Therefore, they can prevent CyA precipitation in the gastrointestinal tract (Gershanik and Benita, 2000). In the presence of the triglyceride sesame oil, the ability to prevent precipitation seems to be reduced. Based on these findings, formulation 2 was excluded from further evaluation.

Upon intravenous administration to rats at a dose level of 10 mg/kg, CyA was characterized by a terminal half-life ($t_{1/2}$) of 11 h, a low clearance (Cl) of 8.6 ml/min/kg, and a high volume of distribution (V_d) of 7.8 l/kg (Table 3). Upon oral administration, the overall bioavailability (F) of CyA in formulation 1 was twofold higher as compared to the microsuspension formulation. The time to reach maximum plasma concentration (t_{max}) and the terminal half-life ($t_{1/2}$) were similar for the microsuspension and formulation 1 with t_{max} values of 6.3 and 7.0 h and $t_{1/2}$ values of 16.7 and 20.5 h, respectively (Table 4, Fig. 1).

4. Discussion

In the current investigation, a self-dispersing formulation capable of increasing the oral bioavailability of CyA as compared to a suspension of

Table 3
Pharmacokinetic parameters of CyA upon intravenous administration to rats as microsuspension at 10 mg/kg; $n=3$

Formulation	$t_{1/2}$ (h)	Cl (ml/min/kg)	V_d (l/kg)
Microsuspension	10.9±1.4	8.6±1.0	7.7±0.5

$t_{1/2}$, half-life; V_d, volume of distribution; Cl, clearance; mean±SEM.

Table 4
Pharmacokinetic parameters of CyA upon oral administration to rats at 20 mg/kg as microsuspension and in the Solutol HS 15-based formulation; $n = 3$

	t_{max} (h)	c_{max} (ng/ml)	$t_{1/2}$ (h)	AUC_{all} (ng/ml h)	F (%)
Microsuspension	6.3±1.7	432.0±18.3*	16.7±4.7	6537.0±438.0**	35.7±3.3***
Formulation 1	7.0±1.0	684.0±73.4*	20.5±2.9	11018.3±423.9**	69.9±2.8***

c_{max}, maximum plasma concentration; t_{max}, time to reach c_{max}; $t_{1/2}$, half-life; AUC, area under the plasma concentration–time curve; F, oral bioavailability. Significance: *$P \leq 0.03$, **$P \leq 0.003$, ***$P \leq 0.001$; mean±SEM.

microparticulate compound was developed. The dosing vehicle was composed of the surface-active ingredients Solutol HS 15, Labrafil M2125CS, and oleic acid. In theory, co-administered excipients could impact the oral bioavailability as well as the overall absorption profile of CyA by a number of different mechanisms. For example, an increase in the intrinsically low aqueous solubility of CyA in the presence of co-administered ingredients may overcome the problem of solubility-limited absorption. From our in vitro solubility study in human gastric and simulated intestinal juice, enhanced solubility of CyA in the gastrointestinal fluids indeed seems to be a major contributor to its comparatively high oral bioavailability in formulation 1. Incorporation of CyA into emulsion droplets or mixed micellar structures may have partly overcome the problem of bile-dependent absorption of CyA.

Additional factors that could have contributed to the improved oral bioavailability of CyA in the self-dispersing formulation would be inhibition of intestinal efflux transporters such as p-gp (Saeki et al., 1993), and/or cytochrome P450 3A (Tjia et al., 1991). For example, Solutol HS 15 has been reported to reverse multidrug resistance in the human carcinoma cell line KB 8-5-11 (Buckingham et al., 1996). However, using primary cultures of porcine brain capillary endothelial cells (PBEC), formulation 1 was identified as a week inhibitor of p-gp with an IC_{50} of 15 μM only (Bravo González et al., 2001). From an in vitro experiment with cytochrome P450 3A4 expressing microsomes prepared from human lymphoblastoid cells, among the three components of formulation 1 only Solutol HS 15 did inhibit to a certain degree cytochrome P450 3A4 ($IC_{50} = 5.6$ μM) (Bravo González et al., 2001). It remains to be elucidated if these in vitro results can be directly translated to the in vivo situation in the rat. A direct extrapolation to the in vivo situation may be difficult for several reasons: first, the in vitro tests cited above have been performed with tissues different from intestinal tissue and in species different from the rat. Second, evaluating surfactant effects in vitro is a difficult task, since their in vivo characteristics are strongly dependent on their actual concentration at the site of action as well as on their accessibility to the tissue. Third, a potential interaction between the surfactant molecules and the test components cannot be excluded. Incorporation of the test compound into micellar structures formed by the surfactants could reduce its accessibility to the site of action.

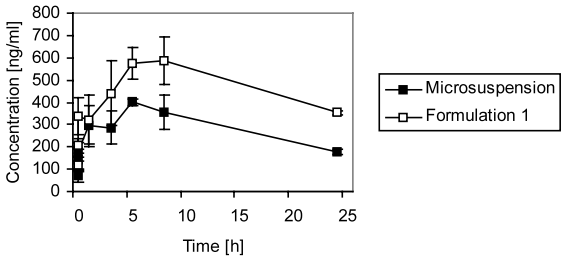

Fig. 1. Plasma concentration–time profile of CyA upon oral administration to rats at 20 mg/kg as microsuspension and in the Solutol HS 15-based formulation (formulation 1); $n = 3$; mean±SEM.

There are some reports retrievable in the literature indicating that both intestinal efflux and metabolism of CyA are saturable processes. For example, it has been shown in vitro that apical to basolateral transport of CyA in Caco-2 cells had a saturable component up to a concentration of 1 μM and that at higher concentrations permeation

increased over-proportionally (Fricker et al., 1996). Moreover, in vivo, an increasing bioavailability of CyA with increasing dose has been observed in rat experiments at dose levels between 3 and 23 mg/kg (Ueda et al., 1984; Lindberg-Freijs and Karlsson, 1994). As in Ueda's study the amount of olive oil in the dosing vehicle varied inversely with the CyA dose, the authors attributed their findings to an olive oil-dependent decreased gastric emptying rate. It was suggested that as a consequence, the extent of gastric mucosal metabolism of CyA had increased at low doses. In Lindberg-Freijs' and Karlsson's study, the composition of the formulations used to administer the various doses did not differ from each other. An effect of the dosing vehicle on gastric emptying rate could, therefore, be excluded. The authors suggested that saturable first-pass metabolism including saturable gut wall metabolism was a major contributor to the increased oral bioavailability with increasing dose. Given Lindberg-Freijs' and Karlsson's hypothesis of saturable first-pass metabolism in the rat holds true, inhibition of CyA metabolism by the co-administered formulation ingredients would eventually not be visible at the high dose level of 20 mg/kg administered in our experiment.

Whenever administering compounds in oil- and surfactant-based excipients, formulation-dependent delays in gastric emptying rate (Meyer et al., 2001) as well as sustained release from the dosing vehicle (Burcham et al., 1997) have to be considered. As a consequence, compared to an 'inert' dosing vehicle, the t_{max} of co-administered compounds would be increased concomitant with a decrease in c_{max}. As CyA is reported to be absorbed particularly in the small intestine (Drewe et al., 1992) and as the molecule is prone to extensive gut wall metabolism (Tjia et al., 1991), sustained release could result in a reduced oral bioavailability. However, when taking into account that the t_{max} of CyA was similar with the two different formulations, altered gastrointestinal motility as well as sustained release from the Solutol HS 15-based dosing vehicle do not seem to have contributed to the difference in the oral bioavailability with the two formulations.

5. Conclusions

The current data demonstrate that we were able to develop an oral CyA formulation based on the amphiphilic excipient Solutol HS 15, suitable to improve its bioavailability in the rat as compared to a microparticulate suspension of the compound. The most likely reason for the enhanced systemic exposure of CyA in the Solutol HS 15-based formulation is the marked solubility increase of CyA in gastrointestinal fluids. In the presence of the surfactants, the bile salt- and pancreatin-dependent absorption of CyA seems to have been overcome. Additional factors that may have contributed to the improved oral bioavailability of CyA in the presence of the surface-active ingredients may be inhibition of intestinal metabolism and/or efflux processes. In order to clarify these issues, the metabolism and transport of CyA in the presence and in the absence of the excipients in formulation 1 will be prospect of further investigations.

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