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International Journal of Pharmaceutics 270 (2004) 109–118
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Intestinal absorption of penclomedine from lipid vehicles in the conscious rat: contribution of emulsification versus digestibility

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Received 21 May 2003; accepted 10 October 2003

Abstract

While the inclusion of highly lipophilic compounds in self-emulsifying drug delivery systems (SEDDS) is often reported to result in strongly enhanced oral absorption, it is still controversial whether further lipolysis of the dispersed lipidic material is required for final transfer to the enterocyte membranes.

In order to assess the relative roles of lipid vehicle dispersion and vehicle digestibility in the oral absorption of penclomedine (Pcm), a series of formulations of Pcm in medium chain triglyceride (MCT)/tocophersolan (TPGS) was developed having three sizes (160 nm, 720 nm, and mm-sized ('crude' oil)); with or without the inclusion of tetrahydrolipstatin (THL), a known lipase-inhibitor.

Oral absorption of Pcm was studied after administration of small volumes of these formulations in the conscious rat. Kinetic evaluation was performed using population analysis. Formulations with particle size 160 nm had the highest relative bioavailability (set at $F = 1$), whereas administration in particle size 720 nm had slightly lower bioavailability ($F = 0.79$). Co-inclusion of THL yielded similar bioavailability for these two SEDDS. 'Crude' oil formulations had $F = 0.62$ (without THL) and 0.25 (with THL).

The data in the current investigation emphasize the prominent role of increased vehicle dispersion relative to digestibility in the absorption of Pcm from MCT-TPGS in submicron emulsions. Only with Pcm administered as undispersed MCT, absorption was more dependent on the action of lipase as bioavailability was inhibited two-fold by the co-incorporation of THL.

Keywords: Bioavailability; Emulsion; Lipolysis; Penclomedine; Pharmacokinetics; Medium-chain triglycerides; Tocophersolan (TPGS)

1. Introduction

Penclomedine (NSC-338720, Pcm) is a highly lipophilic cytotoxic agent which is under active clinical investigation (O'Reilly et al., 2001; Liu et al., 2002). Penclomedine has poor aqueous solubility (<1 μg/ml), good solubility in triglycerides (180–280 mg/ml) and a high octanol/water partitioning coefficient (5.4) (Myers and Stella, 1992). Not surprisingly, its absorption from the crystalline state is poor. An important step forward in improving the oral bioavailability of Pcm can be made by the transition out of the crystal lattice into a molecularly dissolved state. Due to the lipophilic nature of the molecule and its low melting point, lipidic excipients are suitable first choices as vehicles that might improve intestinal absorption. However, the selection and optimization

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doi:10.1016/j.ijpharm.2003.10.036

of lipid excipients is still hampered by the overabundance of possible choices, and on a more fundamental level, by an incomplete understanding of the in vivo fate of these formulations within the gastrointestinal (GI) tract. Substantial variabilities in bioavailability have been documented in kinetic studies when triglycerides of various chain length are selected as lipid phase. Undigestible vehicles as mineral oil have been reported to be associated with lower, but nevertheless substantial absorption indicating that absorptive pathways other than via endogenous lipid digestion can be existent. Other examples are also available where effects of vehicle digestibility on drug absorption were drug-specific (Bloedow and Hayton, 1976).

A number of literature reviews have attempted to identify and categorize the different factors that can affect the processing of the lipid vehicle and incorporated compound in the GI tract in its absorptive route, thereby affecting bioavailability (Charman et al., 1992, 1997; Humberstone and Charman, 1997; MacGregor et al., 1997; Charman, 2000; Gershanik and Benita, 2000; Porter and Charman, 2001).

The most prominent classes of variables identified in most of these reviews are: (1) physicochemical and biopharmaceutical properties of the compound; (2) properties of the lipid vehicle (including the chosen tensioactive compounds and cosolvents); (3) emulsification (also termed lipid vehicle dispersion); and (4) digestibility (also referred to as lipolysability).

In the current investigation, the first two variables are fixed by selecting one drug (Pcm) and one lipid vehicle, consisting of medium chain triglycerides and tocophersolan (TPGS). This allows to study the contributions of emulsification and digestibility on the extent and rate of absorption of Pcm. Emulsification leads to an improved effective surface that can mediate transfer of Pcm to the absorptive epithelium and is represented by route B in Fig. 1. This route, which can be termed interfacial partitioning, comprises at least three subroutes, as Pcm present on the droplet interface can partition to the intestinal absorptive membranes either directly after direct interaction with the enterocyte bilayer, via the aqueous phase, or via transient solubilization in a micellar phase formed by either duodenal components or surfactants from the administered formulation (Myers and Stella, 1992; Charman et al., 1997).

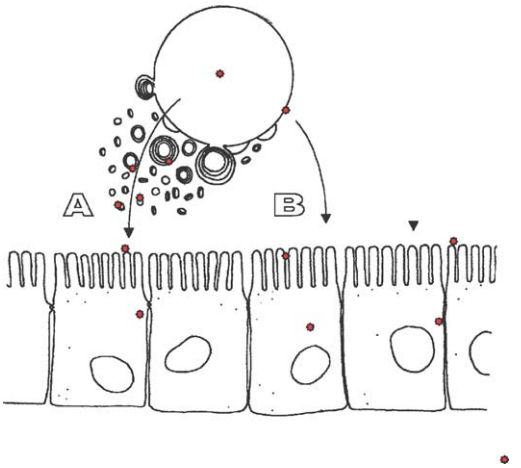

Fig. 1. Intestinal absorptive pathways of Pcm (represented by filled stars) from administered triglyceride emulsion. Route A represents the endogenous lipid digestion pathway in which the action of lipase results in the formation of a ‘product phase’; route B comprises all subroutes involving interfacial partitioning.

Route A in Fig. 1 represents the endogenous lipid digestion pathway in which the action of lipase results in the formation of a ‘product phase’ (Hernell et al., 1990; Staggers et al., 1990). The formation of this product phase is aided by the influx of bile, and liquid crystalline intermediates build up on the surface of degrading lipid droplets. These liquid crystalline structures are believed to be ultimately converted into micelles. Microscopic studies have also provided evidence to support the existence of a so-called ‘hydrophobic continuum’, linking the dispersed and degrading oil droplets to the interior of the product phases (Patton et al., 1985; MacGregor et al., 1997).

The study of the role of digestibility of lipid vehicles in vivo has been hampered by the lack of satisfactory experimental methods. Both pancreatectomy and bile duct cannulation have been reported to result in incomplete inhibition of lipolytic activity, allowing partial lipid digestion (Reymond et al., 1988). In the current studies, THL (tetrahydrolipstatin, orlistat) is utilized as a tool to inhibit lipolysis of the (un)emulsified triglyceride phase. THL is a potent inhibitor of lipases (Borgstrom, 1988; Hadváry et al., 1988) and is known to effectively protect triglyceride droplets when predissolved in sufficient quantities in these droplets (Fernandez and Borgstrom, 1989). Different from classical approaches that interfere with

the incoming flux or activity of lipases, the protecting effect comes from within the droplets. The utilization of THL allows using the same triglyceride oil phase to compare the lipid vehicle effect of a vehicle with intact or without an inhibited lipid digestion pathway. Furthermore, the current studies have been performed with conscious rats in order to avoid the possible depressant effects of general anesthesia on gastrointestinal physiological functions (Healy et al., 1981; Grundy, 1990; Myers and Stella, 1992). A series of formulations of Pcm was developed with increasing degree of emulsification (quantified as particle size) samples of each formulation were administered to conscious rats in the presence or absence of THL.

Finally, pharmacokinetic (pk) modeling of the blood concentration versus time profiles allowed to draw conclusions on the relative relevance of size and digestibility of the utilized lipid emulsions for oral absorption of Pcm.

2. Experimental

2.1. Materials

Pcm (3,5-dichloro-2,4-dimethoxy-6-trichloromethylpyridine, NSC-338720) was supplied by the National Cancer Institute, Bethesda, MD, USA. TPGS (tocophersolan, D-α-tocopheryl polyethyleneglycol 1000 succinate) was obtained from Eastman Chemical Company, Kingsport, TN, USA. Miglyol 812 (MCT, medium chain triglycerides) was purchased from Roig Farma, Terrassa, Spain. THL was purified (>98%) from the commercially available capsules and subsequent analysis was performed by differential scanning calorimetry and HPLC (Wieboldt et al., 1998). Ammonium acetate was from Carlo Erba, Milan, Italy. *t*-Butylmethylether and acetonitrile were purchased from Merck, Darmstadt, Germany. DDT ((1,1-Bis-4-chlorophenyl)2,2,2-trichloroethane) was obtained from Aldrich Chemical Company, Milwaukee, WI, USA.

2.2. Dosage forms

The dosage forms used in this study consisted of a 10% (w/w) oil phase dispersed in water. Two lots of lipid/surfactant phase consisting of medium chain triglycerides (MCT) and TPGS were prepared with 50/50 and 95/5 (w/w) ratios, respectively. Upon cooling, these mixtures solidified but could be rapidly be molten using a microwave or normal oven at 70 °C.

Pcm and, in the indicated preparations, THL were dissolved in the molten mass at 10% (w/w) and 1% (w/w), respectively. Immediately before administration, the lipid phases were emulsified using bidistilled water at a 10% (w/w) lipid/water ratio. The compositions containing the high amount of surfactant required only a short vortex step in order to prepare the emulsions, whereas the dosage forms containing the low amount of TPGS sonicated for 2 min in 30 s increments while cooling (Microson sonifier, Misonix, Farmingdale, NY, USA). The final Pcm concentration in this series of preparations was 10 mg/g. Pcm is reported to be very stable in emulsions (Prankerd et al., 1988), nevertheless care was taken to proceed as rapidly as possible with administration to animals.

Particle size analysis of all emulsions was performed using a submicron particle sizing apparatus (Malvern Zetasizer, Malvern, PA, USA).

2.3. Animal experiments

Male Wistar rats weighing from 250 to 270 g supplied by CIFA (University of Navarra) were used in this study.

All rats were fasted overnight with free access to water. At the day of the experiment, 500 μl of the indicated dosage form was administered as a bolus by oral gavage. At the indicated times, blood samples were obtained under a short ether anesthesia by orbital sinus blood sampling using haematocrit capillaries. Blood was collected in heparinized tubes and immediately frozen and stored at −20 °C until further analysis. In order to compare this experimental protocol, a parallel kinetic study of formulation I was performed by a set of six cannulated rats. Both experimental protocols had the advantage that general anesthesia could be avoided, however, the length of the cannulation experiment (9 h) was associated with substantial technical problems as well as increased suffering by the animals. Animals were cannulated in their jugular artery 1 day before the experiment. Upon administration of formulation I, animals were immobilized in cages, and blood samples were collected in heparinized tubes at the indicated times. Kinetic analysis indicated that the absorption of Pcm from formulation I was identical

(data not shown) for both experimental protocols, and the orbital sinus blood sampling protocol was preferred for the remainder of the studies. The protocols of the studies were approved by the Committee of the animal Experimentation of the University of Navarra.

2.4. Sample preparation and analysis

Sample preparation was a modification of the procedure reported previously (Myers and Stella, 1992). In short, blood samples were thawed, and 100 μl was pipetted into a 15 ml glass tube. An aliquot of internal standard (DDT, in acetonitrile) was added to each sample, followed by 1.0 ml of saline and 5 ml of *t*-butylmethylether. The tubes were then vortexed for 45 s, and the organic layer was removed after a 5 min centrifugation at 850 × *g*. The organic layer was transferred to a new conical glass tube, and evaporated under vacuum. The residue was taken up in 200 μl acetonitrile and analyzed by HPLC.

A 25 μl aliquot was injected into a Hewlett Packard series 1100 HPLC apparatus operating at a wavelength of 243 nm. Separation of Pcm from the matrix and DDT was accomplished by using a Lichrospher 60 RP select B (5 μm, Merck) column with a mobile phase consisting of 80% acetonitrile and 20% 0.05 M ammonium acetate at a flow rate of 1 ml/min. The peak area ratio of Pcm to DDT was determined and compared to the standard curve in order to determine whole blood concentration of the compound of interest. The quantification limit for Pcm, given by a CV of 7.8%, was determined to be 10 mg/l. Calibration curves were prepared with spiked plasma over the range of 10–200 mg/l. The correlation coefficients (*r*) for the calibration curves were >0.997. In the range of the concentrations tested, the mean value of recovery was 95%. with a CV lower than 5%. Within-day CVs were <5.1% and between-day CVs were all <7.9%.

2.5. Data analysis

Individual analysis of the plasma versus time concentration profiles revealed the presence of a flip-flop (Rowland and Tozer, 1995) phenomena preventing the appropriate pk parameter estimation in several animals. To overcome this difficulty it was (reasonably) assumed that differences in drug formulation might modify the rate of absorption and/or bioavailability (*F*), however, drug disposition will remain unaffected and all the data were fitted simultaneously using the population approach. Therefore, there is enough information to discriminate between drug disposition (data from 36 animals) and drug absorption (different from each formulation).

Population analysis uses all available data but preserves individuality, thus the mean and individual profiles can be described. Briefly, the observed drug concentration in the *i*th animal measured at time *j*th (C_{ij}) were modeled as (Sheiner and Grasela, 1991a,b)

$$C_{ij} = f(\mathrm{PK}_i, D_i, t_j) + \varepsilon_{ij} \quad (1)$$

where D_i is the dose given to the *i*th animal, PK_i is the set of individual pk parameters (i.e. total plasma clearance), and f represents the structure of the pk model (i.e. mono-compartmental); ε_{ij} is the difference between the observed value (C_{ij}) and the model predicted value; the set of εs represents a random variable, the residual (intraindividual) variability, and are assumed to be independent and symmetrically distributed around the 0 value with variance equal to σ^2. In (1) an additive residual model is represented; however different residual models such as the proportional, combined error models were also tested.

Individual pk parameters (the components of PK_i) were described as follows:

$$\theta_{1,i} = \theta_{1,\mathrm{pop}} \times \mathrm{e}^{\eta 1,i} \quad (2)$$

where $\theta_{1,i}$ represents the value of the parameter θ_1 in the *i*th animal; $\theta_{1,\mathrm{pop}}$ is the typical value (equal to all rats) of the parameter θ_1, and $\eta_{1,i}$, represents the deviation of $\theta_{1,i}$ from $\theta_{1,\mathrm{pop}}$. The set of η_1 are assumed to be symmetrically distributed around 0 and forms a random variable with variance ω_1^2 reflecting the unexplained interindividual variability associated to the θ_1 parameter. The variances $\omega^2_{1,\ldots,n}$ (*n*, being the number of pk parameters in the model) represent the diagonal elements of the Ω matrix. The goal of the population analysis is to provide robust estimates of $\theta_{1,\ldots,n}$, Ω and σ^2. Results from the population analysis were expressed as parameter estimate and CV% (the standard error for each parameter divided by the parameter estimate multiplied by 100). All the analyses were performed with the NONMEM version V computer program (Beal and Sheiner, 1992). The estimation method used during the model development process was the first-order conditional estimate (Beal and Sheiner, 1999).

Drug disposition were characterized by apparent plasma clearance (CL), and apparent volume of distribution (V) in the case of a mono-compartmental model, and by apparent CL, apparent V_c (volume of distribution of the central compartment), apparent V_{ss} (volume of distribution at steady-state) and apparent intercompartmental clearance (CL_d) for a two-compartmental model. Different drug absorption models were explored: First- and zero-order absorption models, absorption from multiple sites, etc. In addition, the presence of lag time was also tested. For each formulation, relative bioavailability was estimated by setting (arbitrarily) the value of F in group I to 1. Formulation effects were tested in the absorption rate constant, lag time and F.

To select between models the difference in the minimum value of the objective function (OBJF) provided by NONMEM was used. The difference in OBJF between two nested models was compared with a χ^2 distribution in which a difference of approximately 4, 6, and 11 points was significant at the 5, 1, and 0.1% levels, respectively.

In addition to the OBJF test, the precision of the estimates and the analyses of goodness of fit plots served as a guide in the model building process.

3. Results

3.1. Formulation development

In order to investigate the relative roles of lipid vehicle dispersion and vehicle digestibility on the oral absorption of Pcm, a series of formulations with varying particle size, but qualitatively the same excipients, was developed.

Depending on their relative compositions, medium chain triglycerides can form self-dispersing compositions with PEG-tocopherol (tocophersolan, TPGS) with different particle sizes as measured by photon correlation spectroscopy. Compositions of 10% Pcm in MCT/TPGS (50/50%, w/w) form very fine emulsions of ±160 nm upon dilution with water, and co-inclusion of 1% THL slightly increased particle size (Table 1).

Lowering the relative amounts of TPGS allowed to obtain two compositions resulting in Pcm emulsions with an intermediate particle size of ±710–730 nm. Contrary to the first set of formulations (I and II) where vortexing was sufficient for emulsification, composition with low TPGS (III and IV) needed a sonification step. The composition of the latter formulations were taken as a basis for the administration of the crude oil preparations, where the same amounts of oil and surfactant phase were administered, but without the sonification procedure.

Table 1 presents the compositions and mean particle sizes of the administered dosage forms. Mean particle sizes of the preparations were stable over at least 3 h (data not shown). Moreover, the preparations were emulsified immediately before administration.

3.2. Absorption experiments and pk analysis

Fig. 2 shows the mean observed and mean model predicted blood Pcm concentration versus time profiles. Model predictions are those obtained from the selected model (Model 4; Table 2) and describes properly the data set shown in Fig. 2 and the individual

Table 1
Composition and particle size of administered dosage forms

Dosage forms	Composition	Mean particle size administered preparation (nm)	Contains THL?	Anticipated mechanism for intestinal absorption
I	10% penclomedine/45% MCT/45% TPGS	160	−	Interfacial partitioning + lipid digestion
II	10% penclomedine/44.5% MCT/44.5% TPGS/1% THL	180	+	Interfacial partitioning
III	10% penclomedine/85.5% MCT/4.5% TPGS	710	−	Interfacial partitioning + lipid digestion
IV	10% penclomedine/84.5% MCT/4.5% TPGS/1% THL	730	+	Interfacial partitioning
V	10% penclomedine/85.5% MCT (4.5% TPGS)	Unemulsified	−	Interfacial partitioning + lipid digestion
VI	10% penclomedine/84.5% MCT (4.5% TPGS/1% THL)	Unemulsified	+	Interfacial partitioning

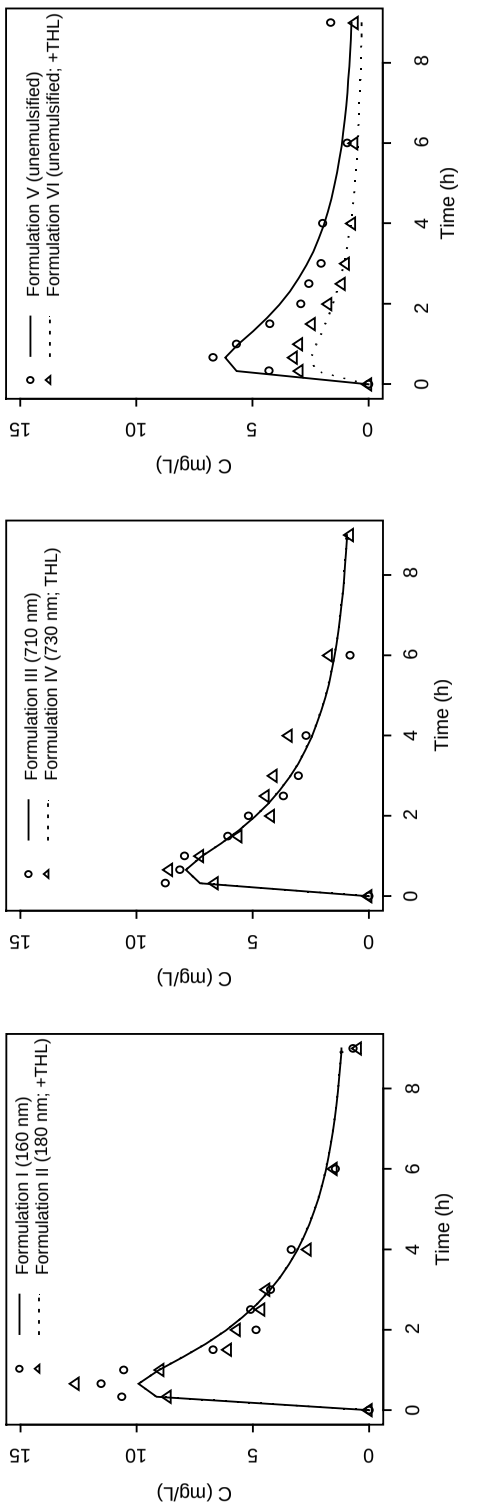

Fig. 2. Mean raw data (symbols) and typical model predicted (lines) Pcm plasma concentrations vs. time profiles. Formulations I–VI correspond to those described in Table 1.

Table 2
Summary of key models fitted to the plasma concentration vs. time data

Model	Model characteristics	OBJF
1	Mono-compartmental; first-order absorption Interindividual variability in V, CL Same F, and K_a for groups I–VI	651
2	Bi-compartmental; first-order absorption Interindividual variability in V_c, CL and CL_d Same F, and K_a for groups I–VI	621 $P < 0.001$
3	Bi-compartmental; first-order absorption Interindividual variability in V_c, CL and CL_d Same F for groups I–VI Different estimate of K_a for each group	614 Five extra parameters with respect model 2 $P > 0.05$
4	Bi-compartmental; first-order absorption Interindividual variability in V_c, CL and CL_d Same K_a for groups I–VI $F_{groups\ I,\ II}$; $F_{groups\ III,IV}$; $F_{group\ V}$; $F_{group\ VI}$	508 $P < 0.001$ Selected model
5	Bi-compartmental; first-order absorption Interindividual variability in V_c, CL and CL_d Same K_a for groups I–VI Different estimate of F for each group	508 $P > 0.05$

V, apparent volume of distribution; V_c, apparent volume of distribution of the central compartment; CL, total apparent plasma clearance; CL_d, apparent intercompartmental clearance; F, relative bioavailability; K_a, first-order rate constant of absorption; OBJF, minimum value of objective function; Groups I–VI correspond to I–VI dosage formulations described in Table 1.

observations (data not shown). Table 2 lists the key models evaluated during the pk analysis. Drug disposition was best described with a two compartmental model ($P < 0.001$), and a first-order absorption model was complex enough to describe the absorption process. The presence of a lag time did not improve significantly the description of the data ($P > 0.05$). Formulation effects were tested in the rate of absorption ($P > 0.05$) and in F ($P < 0.001$). Relative F was found to differ significantly ($P < 0.001$) between groups I–II, groups III–IV, groups V and VI (model 4; Table 2); however, differences in F between groups I and II and between groups III and IV were found to be statistically irrelevant ($P > 0.05$; model 5; Table 2). Estimates (CV) obtained for F in model 5 were 1 (fixed), 0.94 (26), 0.71 (22), 0.78 (20), 0.60 (27), 0.24 (18) for groups (formulations) I–VI, respectively. Interindividual variability was included in V_c, CL and K_a (first-order rate constant of absorption) ($P < 0.001$) but not in V_{ss}, CL_d and F ($P > 0.05$). Residual variability had an estimate of 23%. Parameter estimates obtained from model 4 are shown in Table 3.

Table 3
pk parameter estimates

Parameter	Estimate	Interindividual variability
CL (l/h)	0.086 (24)	37 (54)
V_c (l)	0.38 (25)	48 (53)
K_a (h^{-1})	4.65 (11)	NE
$F_{I,II}$	1 (fixed)	
$F_{III,IV}$	0.79 (15)	NE
F_V	0.62 (22)	
F_{VI}	0.25 (15)	
CL_d (l/h)	0.08 (37)	90 (67)
V_{ss} (l)	1.2 (38)	NE

Estimates of interindividual variability and parameter precision in parenthesis are expressed as coefficient of variation: V_c, apparent volume of distribution of the central compartment; CL, total apparent plasma clearance; CL_d, apparent intercompartmental clearance; F, relative bioavailability; K_a, first-order rate constant of absorption; V_{ss}, apparent volume of distribution at steady-state; I–VI refers to the I–VI dosage forms described in Table 1; NE, interindividual source of variability not significant and therefore not estimated.

4. Discussion

The intent of the current studies was to assess the relative roles of emulsification and digestibility in the oral absorption of a highly lipophilic compound, Pcm, from lipidic formulations. The first extensive and excellent study of lipid vehicle effects on the oral absorption of Pcm has been communicated by Myers and Stella (1992). These authors compared oral absorption of Pcm from a series of emulsions using tributyrin, trioctanoin, triolein, soybean oil and mineral oil as oil phases. Bioavailability was measured after duodenal infusion of emulsions of a relatively small volume (±0.5 g) in anaesthetized rats.

In the present studies, two components were selected for the design of a set of formulations that were addressing this theme: MCT as the primary oil phase and TPGS as emulsifier. Only their relative compositions were varied in order to obtain emulsions with defined particle sizes. TPGS has the additional property that it is a potent inhibitor of Pgp (Yu et al., 1999) which is a further factor that might affect intestinal absorption studies and possibly blur the interpretation of experimental results. It has long been known that the choice of the triglyceride can influence the absorptive process in the intestine, for instance by promoting the formation of chylomicrons, followed by further routing in the lymph, as well as solubility effects of the digested food digest products. Upon the action of lipase, tributyrin will release highly soluble short chain fatty acids with a poor capacity to solubilize dissolved lipophilic compounds and Pcm is anticipated to precipitate in situ in the GI-tract, impairing absorption (Myers and Stella, 1992). Arachis oil has been shown to promote the lymphatic uptake of DDT as long chain unsaturated and saturated fatty acids form colloidal intermediate phases with incoming bile (Palin et al., 1982). MCT is reported to promote absorption through the portal route (Palin et al., 1982; Nankervis et al., 1996). Whereas Palin et al. suggested that the fatty acids released upon lipolysis of MCT are sufficiently water-soluble for intestinal absorption without the necessity for solubilization by biliary material (Palin et al., 1982), other authors indicated that hydrolysis products of the related trioctanoin are incorporated into biliary micellar phases (Rautureau and Rambaud, 1981; Myers and Stella, 1992).

General anesthesia is known to be associated with modified gastrointestinal motility (Healy et al., 1981; Grundy, 1990; Myers and Stella, 1992). As this study intended to investigate the role of endogenous lipid digestion, possible interference with this process was avoided and instead, a conscious rat model was chosen (although a short anesthesia for the ocular punction had to be applied). Furthermore, a limited amount of lipidic material was administrated. It has been reported that non-physiological volumes of lipidic vehicles are associated with altered intestinal metabolism (Humberstone and Charman, 1997). The oral and intravenous kinetics of Pcm have been described in the previous study of Myers and Stella, and indicated that about 8% of the incomplete bioavailability in their rat model might be accounted for by a possible first pass effect (Myers and Stella, 1992). As the current studies have focussed on relative rather than absolute bioavailabilities, intravenous as well as lymphatic absorption studies were omitted.

A series of three formulations consisting of Pcm, MCT and TPGS and varying in particle size (160–180; 710–730 nm and crude oil) were developed. In order to assess the influence of digestibility, each set was divided into a formulation with or without THL. THL is a known inhibitor of intestinal lipases and is reported to effectively protect triglyceride droplets when predissolved in oil in the concentrations used in this study (Fernandez and Borgstrom, 1989). It was anticipated that when digestion of lipid is essential for uptake of Pcm, co-inclusion of THL would result in low or nonexistent bioavailability.

The experimental data clearly indicate that the emulsified formulations containing THL all had similar F values as their analogous compositions without THL, providing no support for a critical role of lipid digestion for the absorptive pathway of Pcm in this study. Instead, the finest emulsions of below 200 nm had identical blood-time profiles for the preparations with and without THL, suggesting that interfacial partitioning dominated the absorptive process.

The lipid particles of intermediate size (formulations III and IV, 710–730 nm), appeared to have slightly lower bioavailabilities ($F_{\mathrm{III,IV}} = 0.79$ relative to $F_{\mathrm{I,II}} = 1$). Furthermore, lower maximum blood concentration values were obtained as compared to formulations I and II. The data suggest that for lipid particles of these dimensions, interfacial transfer can

still result in substantial absorption. Administration of Pcm in crude MCT oil resulted in significantly lower *F*, and furthermore showed a quantitative (two-fold decreased absorption) effect of the inhibition of the lipid digestion pathway. For Pcm dissolved in crude oil, the diminution of particle size attained by mechanical forces in the GI tract, such as the passage through the pylorus (Carey et al., 1983) is not sufficient for the generation of an absorptional surface that can yield efficient interfacial transfer. In this case, the lipid digestion pathway substantially aids the absorptive process.

It might be speculated that the apparent higher absorption rates of Pcm from soft gel capsules containing a solution of Pcm in Neobee 1053 (comparable to MCT) in humans (O'Reilly et al., 2001; Liu et al., 2002), might point towards a dimensional issue. In the present studies, the administered volume of 0.5 ml was substantial relative to the dimensions of the GI tract of the rat as compared to the human situation, where the applied size 11 capsule is much smaller in comparison with the lumen of the human GI tract. This might result in an improved ability of the human GI tract to process the capric/caprylic triglycerides, leading to somewhat higher bioavailabilities.

The importance of the interfacial transfer (pathway B, Fig. 1), also termed diffusional process in other reports (Myers and Stella, 1992; Humberstone and Charman, 1997) has been stressed in the literature. On the other hand, several authors have concluded that release from the administered oil due to digestion was important for absorption (Yamahira et al., 1979; Vetter et al., 1985). MacGregor et al. (1997) suggested that lipolysability of SEDDS as an important prerequisite for absorption. In vitro data indicated that there exist qualitative differences in the chosen surfactant that may impair the action of lipase (MacGregor et al., 1997). However, no in vivo studies were provided that correlate the lipolysis of oily formulations with bioavailability from the GI tract.

The data in the current investigation emphasize the prominent role of increased vehicle dispersion in the absorption of Pcm from MCT emulsions. The smaller the lipid particle, the smaller will be the impact of digestion of the lipid droplet. With lipid particles of 150 nm, there is no measurable effect of lipolysis on either extent or rate of absorption of Pcm. MCT particles of this size do not need to be digested by lipase in order to deliver Pcm to the intestinal epithelium. Intermediate size MCT/TPGS emulsions of 710–730 nm still had a dominating effect of interfacial transfer, with lipolysis having at best a modulating effect on the rate of absorption. In contrast, With Pcm administered as crude MCT oil, absorption (which in general was significantly lower) was more dependent on the action of lipase as bioavailability was inhibited two-fold by the co-incorporation of THL.

Future studies are anticipated that will investigate the influence of lipophilicity of the studied drug on the absorptive pathways after administration in lipid vehicles. Some authors have documented effects of $\log P$ on gastrointestinal when analogous compounds with $\log P$ values in the range of 5–8 were administered (Nankervis et al., 1996; Iwanaga et al., 2000). While the aqueous solubility of Pcm ($<1\ \mu g/ml$) apparently does not impair interfacial transfer, it remains to be investigated if compounds with even lower aqueous solubilities will behave similarly. Furthermore, the effect of particle size on the relative contributions of interfacial transfer and lipolysis can be investigated in further detail, possibly allowing to define limits within which formulations become independent of lipolytic processing.

Acknowledgements

This research project was supported by the Department of Education and Culture, Gobierno de Navarra, Pamplona, Spain (2001–2002). Miss J. Kloeckner is thanked for expert assistance in HPLC sample analysis.

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