

Clinical studies with oral lipid based formulations of poorly soluble compounds

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Abstract: This work is an attempt to give an overview of the clinical data available on lipid based formulations. Lipid and surfactant based formulations are recognized as a feasible approach to improve bioavailability of poorly soluble compounds. However not many clinical studies have been published so far. Several drug products intended for oral administration have been marketed utilizing lipid and surfactant based formulations. Sandimmune® and Sandimmune Neoral® (cyclosporin A, Novartis), Norvir® (ritonavir), and Fortovase® (saquinavir) have been formulated in self-emulsifying drug delivery systems (SEDDS). This review summarizes published pharmacokinetic studies of orally administered lipid based formulations of poorly aqueous soluble drugs in human subjects. Special attention has been paid to the physicochemical characteristics of the formulations, when available and the impact of these properties on the in vivo performance of the formulation. Equally important is the effect of concurrent food intake on the bioavailability of poorly soluble compounds. The effect of food on the bioavailability of compounds formulated in lipid and surfactant based formulations is also reviewed.

Keywords: lipid formulations, poorly soluble compounds, pharmacokinetics, human clinical studies, lipophilic compounds, SEDDS, emulsions, food effect

Introduction

The purpose of this article is to review the clinical trials on lipid and surfactant based formulations of poorly soluble compounds for oral administration. Despite a plethora of articles dealing with orally administered lipid based formulations of poorly soluble compounds, the majority of these articles emphasize issues related to pharmacology and toxicity rather than formulation issues. Therefore special attention has been paid to the differences in the pharmacokinetics upon oral administration of different lipid formulations.

The use of lipid and surfactant based formulations is one of several approaches that has been applied in order to improve the oral bioavailability of poorly aqueous soluble compounds intended for oral administration. The approach has proved efficient and hence has received attention, especially in academia. Lipid and surfactant based systems are physically very different systems including systems like emulsions, microemulsion, self-emulsifying drug delivery systems (SEDDS), micellar solutions, and dry emulsions like lipid solutions and lipid suspensions.

The number of excipients which are pharmaceutically acceptable and applicable in the formulation of lipid and surfactant based systems is large. The use of solubilizing excipients in marketed formulations of poorly soluble compounds has been reviewed recently (Strickley 2004).

The bioavailability enhancing properties of lipid and surfactant based systems has most often been attributed to the ability of the vehicles to keep the compound in solution in the gastrointestinal (GI) tract and thereby maintaining a maximal free drug concentration (Porter and Charman 2001). However the underlying mechanisms of

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absorption from lipid and surfactant based systems is not fully understood.

The majority of the knowledge of the *in vivo* performance and suggested mechanism of drug absorption from lipid based formulations originates from studies in animals. Several authors have attempted to identify and describe the important parameters in order to set-up rational strategies for development of new lipid based formulations. The parameters that have been emphasized so far are the degree of emulsification (in terms of particle size), and the solubility of the drug both in the digested and nondigested formulation of the resultant dispersion (Humberstone and Charman 1997; Pouton 2000; Porter and Charman 2001; Porter et al 2004; Nielsen et al 2007).

Furthermore a number of typically employed excipients are susceptible to enzymatic degradation in the GI tract. Excipients susceptible to degradation include natural di- and triglycerides as well as some commonly used surfactants like Labrasol, Labrafil, Gelucire, Cremophor, Tween 80 (Khossravi et al 2002, Seebaluck et al 2004, Larsen et al 2006).

The release of compound from SEDDS based formulation is thought to take place by two major pathways: Interfacial transfer and degradation of vehicle (de Smidt et al 2004; Porter et al 2004). Interfacial transfer can be described as a concentration gradient driven process in which the compound diffuses from the formulation into the bulk or directly over the intestinal membrane. The rate and extent of interfacial transfer is thought to be governed by partition coefficient and solubility in the donor (formulation) and recipient phase particle size and hence surface area of formulation (Armstrong and James 1980). The second pathway is degradation of the vehicle inducing the release of the compound out of the vehicle. As mentioned above, for lipid based formulations, the most important degradation is the lipolysis catalyzed by pancreatic lipase. The release rate is thought to be dependent on the solubility of the compound in the formulation and rate and extent of the degradation of the vehicle. Lipolysis of triacylglycerols (TG) by the pancreatic lipase–colipase complex releases monoacylglycerols, diacylglycerols and free fatty acids. These lipolysis products are amphiphiles that will further assist the solubilization of poorly soluble compounds in the GI fluids.

Recently it has been demonstrated that a number of excipients should be considered as more than just inert substances. It has been shown that Cremophor EL, Tween 80, Labrasol, Miglyol polyethoxylated, can inhibit the PGP efflux transporter (p-glycoprotein) (Hugger et al 2002; Shono et al 2004; Cornaire et al 2004) and hereby potentially improve

bioavailability of drug molecules being PGP substrates. A number of excipients have shown to influence the lymphatic transport both in rats, and have an impact to the chylomicron secretion in caco-2 cells, which is also believed to be an indicator of lymphatic transport (Rege et al 2002; Seebaluck et al 2003; Karpf et al 2004).

These advances in the characterization of the mechanisms underlying the bioavailability enhancing properties of lipid and surfactant based formulations mark a change in formulation strategy away from the more empirically based formulation testing to a more rational formulation strategy. However it also implies that more work is needed in the field and that a battery of different methods should be used to describe the different mechanisms.

Among the lipid based formulations used for oral delivery, SEDDS and self-microemulsifying drug delivery systems (SMEDDS) have been characterized more systematically from a physicochemical point of view. They are isotropic mixtures of oil (pure triglyceride oils, mixed glycerides), surfactant (hydrophilic or/and lipophilic), water soluble co-solvents and the poorly soluble compound. Development and characterization of SEDDS and SMEDDS has been extensively reviewed (Pouton 1997; Gershanik and Benita 2000; Gursoy and Benita 2004; Pouton 2006). A number of biophysical techniques have been used to characterize these systems (Lawrence and Rees 2000). Scattering techniques such as dynamic light scattering (DLS) (Constantinides and Scarlet 1997), small angle neutron scattering (SANS) (Bergenholtz et al 1995) and small angle x-ray scattering (SAXS) (Regev et al 1996) can offer useful information for the structure of the microemulsions together with freeze fracture electron microscopy (Vinson et al 1991). Dielectric viscosity, and conductivity measurements can offer new insights on the characterization of these systems on a macroscopic level (Acosta et al 1996).

Equally important are the interactions between drugs and food. The impact of food on the bioavailability of drugs in clinical studies has recently been reviewed by Schmidt and Dalhoff (2002). Food delays the gastric emptying rate and induces secretion of bile and pancreatic juices, while the passage time of the small intestine remains virtually unchanged. Hence food intake can increase the solubilization time and increase the solubility of a poorly soluble drug, which will affect the pharmacokinetic parameters.

In the following, data from clinical studies of oral lipid based formulations of poorly soluble compounds performed are presented. Table 1 summarizes the type of study and the formulations used.

Table 1 Physicochemical properties and pharmacokinetic data of drugs and formulations used in bioavailability studies with human subjects

Compound	Study design	Formulation composition	Formulation characteristics	Comments	Authors	Year
Atovaquone (LogP 3.68) ¹	Randomized balanced three-way crossover design (n = 9)	Tablets Aqueous suspension An oil suspension	Oil suspension consisted of miglyol (fractionated coconut oil)	AUC: Oil suspension ~aqueous suspension > tablets C _{max} : Oil suspension ~aqueous suspension > tablets	Rolan et al	1994
Clomethiazole (LogP 2.29) ¹	Cross-over study (n = 10)	Tablets Lipid mixture Aqueous mixture	Lipid mixture contained increasing amounts of arachis oil Aqueous suspension contain the corresponded acid form	Plasma concentrations: Aqueous suspension > lipid mixture > tablets. A doubling in the dosed arachis oil resulted in higher plasma concentrations.	Fischler et al	1973
Cyclosporine (log P = 2.92) ²	12 fasted healthy volunteers, four way cross-over	Sandimmune ³ The microemulsions (B&D) were composed of polyethylene glycol, hydrogenated castor oil, middle chain triglycerides and low molecular weight glycols. The solid micellar solution was composed of sucrose monolaurate and propylene glycol.	150 mg CsA from either Sandimmune or B, C or D. B: Microemulsion with fast in vitro release. C: Solid micellar solution with fast in vitro release. D: Microemulsion with slow in vitro release.	AUC: B ~ C > D ~ Sandimmune C _{max} : B ~ C > D ~ Sandimmune T _{max} : B < C ~ D ~ Sandimmune	Drewe et al	1992
	24 fasted healthy volunteers, three way cross-over	Neoral ³	180 mg CsA from Neoral as soft gelatine capsule or as solution mixed with water or juice	T _{1/2} : Mixed with juice ~ Mixed with water < Soft gelatine capsule. All other PK parameters equal.	Kovarik et al	1993
	24 fasted healthy volunteers, two way cross-over with replication	Neoral and Sandimmune	180 mg CsA (Neoral) and 300 mg CsA (Sandimmune) (A)	AUC: Neoral ~ Sandimmune C _{max} : Neoral > Sandimmune T _{max} : Neoral < Sandimmune T _{1/2} : Neoral < Sandimmune Neoral exhibit lower intrasubject and intersubject variability (%CV) than Sandimmune.	Kovarik et al	1994
	24 healthy volunteers, four way cross-over, fasted or with a fat-rich meal	Neoral and Sandimmune	180 mg CsA (Neoral) and 300 mg CsA (Sandimmune)	Fasted AUC: Neoral > Sandimmune. Fed AUC: Sandimmune > Neoral. Fasted C _{max} : Neoral > Sandimmune. Fed C _{max} : Neoral ~ Sandimmune. Fasted and fed T _{max} : Neoral < Sandimmune	Mueller et al	1994b

(Continued)

Table 1 (Continued)

Compound	Study design	Formulation composition	Formulation characteristics	Comments	Authors	Year
	48 fasted healthy volunteers, two parallel study groups, four way cross-over	Neoral and Sandimmune	200 mg to 800 mg CsA as Neoral or Sandimmune	Neoral exhibit linear bio availability in the dose range 200 – 800 mg but Sandimmune did not.AUC: Neoral 174% to 239% higher than Sandimmune.	Mueller et al	1994a
	23 healthy volunteers fed a fat-rich breakfast 30 minutes prior dosing, two-way cross-over	Neoral and Sandimmune	7.5 mg/kg CsA from Neoral and Sandimmune	AUC: Neoral ~ Sandimmune C_{max} : Neoral > Sandimmune	González-Llaven et al	1999
	24 healthy volunteers open randomized 6 healthy volunteers	SMEDDS and non-SMEDDS capsules Lipospheres,	200 mg single oral dose of each treatment 200 mg of cyclosporin, lipospheres containing Tween 80, Span 80, Chremophor RH 40, phospholipids, Tricarpin. Size from 25–400 nm	AUC SMEDDS > AUC non-SMEDDS Higher AUC and C_{max} with lipospheres with small diameter	Postolache et al Bekerman et al	2002 2004
	Fasted healthy volunteers (three in prediction study and six in confirmatory study), open cross-over study	Galactolipid SEDDS (G-SEDDS) and Neoral	300 mg CsA from Galactolipid SEDDS and Neoral, Galactolipid SEDDS particle size of 16–20 μ m (volume median diameter)	AUC: Neoral > G-SEDDS C_{max} : Neoral ~ G-SEDDS T_{max} : Neoral ~ G-SEDDS	Odeberg et al	2003
Danazol (logP = 4.53) ⁴	Randomized crossover fed/fasted study (n = 11 females)	Danocrine capsule O/w-emulsion	Emulsion contained glycerol mono-oleate and was physical stable for 6 months.	AUC: Capsule _(fed) > Capsule _(fasted) Emulsion ~ emulsion _(fasted)	Charman et al	1993
Diazepam (logP 2.84) ¹	Crossover, 2 weeks washout (n = 4), fasted	Tablets Lipid solution	Lipid solution contained MCT	No significant difference in plasma diazepam levels between tablets or lipid solution. However lipid solution was less variable.	Yamahira et al	1979
Dicumarol (logP of 3.66) ¹	Human (n = 4), fasted	Redispersed freeze-dried drug-milk, liquid,	Capsules containing 300 mg drug particles (49 μ m) and 200 ml milk	Statistically significant differences were found between area under the curve, maximum plasma concentration with dicumarol-milk formulation.	Macheras et.al (a)	1986
Flufenamic acid (logP 3.98) ¹	Random design 15 days washout (n = 9)	IR dosage form Lipid mixture	Lipid mixture contained vegetable oil hydrogenated vegetable oil, beeswax and soy lecithin	Mean plasma concentrations: Lipid mixture > IR dosage form at 90 min	Angellucci et al	1976
Griseofulvin (logP 0.68) ¹	Random crossover (n = 5)	Lipid mixture Aqueous suspension Tablets	Lipid mixture contained corn-oil in water emulsions with dispersed drug	Bioavailability determined via urinary excretion: Lipid mixture > tablets ~ aqueous suspension. Absorption increased with increasing lipid amounts and reached plateau with 6 g of lipid	Bates et al	1975
	Four-way crossover study (latin square design) (n = 4)	O/w-emulsion Tablets	O/w-emulsion contained 2, 4, 6 or 12 g of corn oil	Bioavailability determined via urinary excretion: o/w-emulsion ~ tablets Absorption was significantly decreased upon 2 g corn oil in the o/w-emulsion compared with the higher lipid amounts.	Bates et al	1977

4-Hydroxyphenyl retinamide (Fenretinide) ¹ (LogP 5.50)	Humans ⁶	Lipid matrix (LXS) Capsule formulation	LXS (lymph-X-Sorb™) is an organized lipid matrix that consists of lyso-PC, MG and FA	Equivalent peak plasma concentrations were obtained using LXS at 20% of the capsular compound amount.	http://www. avantilipids. com/LXS-DD.asp	2003
Nitrofurantoin (LogP not computable) ¹	Human n = 4 fasted	Redispersed freeze- dried drug- milk, liquid,	Capsule containing 100 mg drug particles (75 µm) and 200 or 400 ml milk	Urine data revealed superiority of the nitrofurantoin-milk formulations regenerated with 200 and 400 mL of milk over the corresponding capsule	Macheras et al (b)	1986
Paclitaxel (LogP not computable) ¹	Randomized design, 2 weeks washout (n = 6)	Cremophor EL solution Polysorbate 80 solution	Both solutions contained ethanol. Prior to administration, all patients received cyclosporine to inhibit the P-gp efflux transporter.	AUC and C _{max} : Polysorbate > Cremophor EL solution	Malingré et al	2001
Progesterone (LogP 3.78) ¹	3 days washout (n = 7)	Plain-milled IR Micronized IR Plain- milled in LCT Micronized LCT	LCT was a mixture of natural oils that were high in long-chained unsaturated FA	C _{max} : micronized progesterone in LCT > all others T _{max} : micronized progesterone in LCT < all others	Hargrove et al	1989
Quingestron (LogP 4.91) ¹	1 week washout (n = 1, each administration repeated 1–4 times) Fed/fasted	Oil suspension Oil solution powder	Oil suspension in sesame oil (10 µm particle size) Oil solution in sesame oil	Bioavailability estimated by urinary excretion (fasted state): oil solution > oil suspension > powder. Fed state increased bioavailability.	Bruni et al	1970
Q10 (Ubiquinone) (LogP not computable) ¹	Four way randomized cross-over (n = 10)	Oil solution Oil and Tween Oil, Tween and lecithin	Oil was soybean oil. Different amounts of oil and surfactants were administered.	AUC: oil solution > other lipid formulations	Weis et al	1994
Retinyl palmitate (LogP 11.0) ¹	1-year; randomized, double blind controlled trial (n = 78 with cystic fibrosis)	Organized lipid matrix (OLM) TG-based nutritional supplement	OLM has a melting point of 64.6 °C, CMC = 0.1 mM and consists of Lym-X-Sorb (lyso-PC, MG, FA in 1:4:2)	AUC: OLM > TG-based nutritional supplement in cystic fibrosis patients	Lepage et al	2002
Saquinavir (LogP 2.73) ¹	Randomized, placebo-controlled, double-blind, four phase cross-over: 1 week washout (n = 8) Open-label, randomized, 2 × 2 crossover study (n = 24) HIV-patients HIV-patients, n = 13	Invirase® with 0, 100, 1000 or 5000 mg Cremophor Fortovase® Invirase® co-dosed with ritanovir Fortovase® Invirase® co-dosed with ritanovir	Fortovase® contain medium chain MG and DG Fortovase® contain medium chain MG and DG	C _{max} and AUC: Cremophor EL dose dependently increase in C _{max} and AUC of saquinavir AUC: Invirase® + ritanovir > Fortovase® + ritanovir AUC: no significant difference	Martin- Facklam et al Kurowski et al Cardiello et al	2002 2003 2003

(Continued)

Table 1 (Continued)

Compound	Study design	Formulation composition	Formulation characteristics	Comments	Authors	Year
α -tocopherol (LogP 7.98) ¹	2-sequence cross-over study, 1 week washout (n = 6)	Solid dispersions Oil solution	Solid dispersion contains Gelucire 44/14 that consists of pegylated FA-esters and glycerides. DSC, Confocal microscopy	AUC: Gelucire 44/14 > oil solution	Barker et al	2003
Tocotrienols (LogP 8.56) ¹	Three way cross-over (n = 6)	SEDSS 1 (coarse) SEDSS 2 (fine) Oil solution	SEDSS 1 produced a droplet diameter of 10.6 μ m, SEDSS 2 of 1.5 μ m	AUC: SEDSS 1~SEDSS2>oil solution T _{max} : SEDSS2<oil solution C _{max} : SEDSS1~SEDSS2>oil solution AUC: SEDSS> soft gelatin	Yap and Yuen	2004
Vitamin E	Two way cross-over study n = 8	Soft gelatine/commercially available SEDSS	SEDSS (Tween, Span, palm oil)		Julianto et al	2000
Vitamin D (logP 7.04) ¹	Fed/fasted, n = 24	Oil solution (LCT) Oil solution (MCT)	LCT was peanut oil and MCT was Miglycol 812®	AUC: LCT oil solution (fasted) > MCT oil solution (fasted) LCT oil solution (fed) ~ MCT oil solution (fed)	Holmberg et al	1990

Notes: 1LogP values calculated using ChemDraw® Ultra version 8.0.3 (CambridgeSoft, USA); 2el Tayar et al 1993; 3Composition and formulation characteristics of Sandimmune and Neoral are described in the text; 4Bakatselou et al 1991; 5See www.emea.eu.int/humandocs/PDFs/EPAR/Forovase/093398en4.pdf; (A) 300 mg CsA from Sandimmune and 150 mg CsA from Neoral was selected to provide comparative exposure of CsA; 6The number of subjects participated in this study is not stated.

For practical reasons the studies presented in the current work have been categorized based on the physicochemical characteristics (oil, suspensions, emulsions, SEDDS, and surfactant based systems). Furthermore the studies on the effect of food to oral bioavailability in humans have been reviewed.

Oil solutions

Lipid based formulations can enhance the bioavailability of poorly soluble drug substances by keeping the compound in solution. Medium chain triacylglycerides (MCT) and long chain triacylglycerides (LCT) are commonly used for the lipid based formulations. Several major differences exist between MCT and LCT in respect with their in vivo fate, such as lipolytic products, differences in the modulation on gastric emptying (Hunt and Knox 1968) and the contraction of the gallbladder in humans (Ladas et al 1984). Long chain lipolytic products delay gastric emptying and facilitate the contraction of the gallbladder to a larger extent than the medium chain lipolytic products (Hunt and Knox 1968).

The bioavailability of quingestrone (progesterone 3-cyclopentyl enol ether belonging to the enol ethers of Δ^3 -kerosteroids) after oral administration to human subjects was evaluated by measuring the urinary excretion of pregnanediol and allopregnanediols (Bruni and Galletti 1970). 100 mg of the compound prepared either as micronized powder (with particle size less than 10 μ m), an oil suspension in 0.3 ml of sesame oil (with particle size less than 10 μ m), or an oil solution in 2.5 ml of sesame oil. Bioavailability of quingestrone was determined as the total urinary secretion for both metabolites. The urinary excretion was highest when the oil solution (6.43 \pm 0.18 mg was administered compared with the oil suspension (2.96 \pm 0.27 mg) or and the micronized powder (0.91 \pm 0.02 mg).

The relative bioavailability of clomethiazole (base form) was investigated after oral administration of two arachis oil formulation both in capsules and a tablet formulation to fasting healthy volunteers (Fischler et al 1973). Higher plasma concentrations were obtained from the oil filled capsules and the absorption was more rapid, compared with tablets. The two arachis oil formulations consisted of clomethiazole and arachis oil 1:1 (384 mg + 384 mg) or 1:2 (384 mg + 768 mg) on a weight basis, respectively. Increasing the arachis oil to clomethiazole ratio from 1:1 to 2:1 resulted in a 1.5 times increase in C_{max} suggesting an increase in bioavailability with increase of co-administered oil amount. Doubling the clomethiazole dose, but keeping the oil: drug ratio at 1:1 resulted in a 1.7 times increase

compared with a tablet formulation indicating nonlinear absorption kinetics. The results emphasizing the impact of the solubilization capacity of the lipid vehicle to the overall performance of the formulation

A soft capsule contained 200 mg of flufenamic acid (analgesic anti-inflammatory agent), 100 mg of vegetable oil, 40 mg of hydrogenated vegetable oil, 8 mg of beeswax and 5 mg of soya lecithin and a hard gelatin capsules contained 200 mg flufenamic acid and 20 mg of magnesium stearate were orally administered to healthy volunteers (Angelluci et al 1976). The plasma levels more than doubled 90 minutes after administration of the lipid solution compared with the powder formulation. However after 180 and 360 minutes the plasma levels were lower after administration of the soft capsules. Due to the low number of plasma samples, it was not possible to identify any significant differences. The different plasma curves observed for the two pharmaceutical forms can be attributed to physicochemical factors, triggered by excipients present in the soft gelatin capsule which result in a faster absorption of the drug.

The absorption of diazepam (benzodiazepine derivative) in fasted human subjects when dosing 5 mg in either a medium chain triglyceride formulation (2% diazepam-MCT solution) or a commercially available tablet formulation (Serenzin®, Sumitomo Chemical Co) in a crossover study was investigated by Yamahira and colleagues (1979). There was no significant difference in the average plasma profiles arising from the two formulations. The authors mainly attributed this to the large intersubject variation and the limited number of subjects ($n = 4$) enrolled in the study. However upon repeated administrations, the MCT solution showed less intrasubject variation. The authors suggested that MCT solution of diazepam produced a more uniform drug absorption rate compared with the tablets for individual patients.

The impact of the physical characteristics of a formulation has been demonstrated by Hargrove and colleagues (1989) in a clinical study with progesterone. Progesterone is indicated for use in the prevention of endometrial hyperplasia in nonhysterectomized postmenopausal women. Four different preparations were made: plain milled progesterone, micronized progesterone, plain-milled progesterone in oil, micronized progesterone in oil. Suspending the micronized progesterone in oil more than doubled the C_{max} , compared with micronized powder, while there was no effect of suspending the nonmicronized progesterone in oil. These results emphasize the effect of concomitant lipid digestion in dissolution of drug, at the same time also showing that the particle size of the solid drug is important.

The effect of medium chain triglyceride was studied by Rolan et al in 1994. In this approach, absorption of 500 mg atovaquone (an antiprotozoal agent) from either a suspension in 30 ml of Miglyol (fractionated coconut oil, medium chain triglyceride), an aqueous suspension using 0.25% w/v methylcellulose as suspending agent, or two 250 mg tablets of atovaquone were used. The AUC values were at the same levels for the aqueous and oil suspensions ($144 \pm 48 \mu\text{g/ml}^{-1} \text{ hr}$ and $144 \pm 124 \mu\text{g/ml}^{-1} \text{ hr}$ respectively), the C_{max} increased for both formulations ($2.7 \pm 1.1 \mu\text{g/ml}$ for the aqueous suspension and $2.0 \pm 1.8 \mu\text{g/ml}$ for the oil) compared with the tablets ($1.1 \pm 0.6 \mu\text{g/ml}$). The higher absorption of atovaquone from the aqueous and oil suspensions compared with tablets was attributed to a better dispersion, allowing a faster solubilization of the drug.

Two oil solutions containing long chain and medium chain triglycerides with vitamin D were administered in human volunteers (Holmberg et al 1990). Higher AUC values obtained for the LCT solution (peanut oil) compared with the MCT solution (Miglyol 812) suggesting that the presence of long chain fatty acids enhance the absorption of vitamin D.

In four out of seven studies the presence of long chain triglycerides (LCT) significantly increased the oral absorption of the drugs (Bruni and Galletti 1970; Fischler et al 1973; Hargrove et al 1989; Holmberg et al 1990). Furthermore LCT formulations demonstrated higher oral absorption compared with MCT ones (Holmberg et al 1990). In contrast no significant difference in the absorption observed among oil formulations containing medium chain triglycerides MCT and tablets (Yamahira et al 1979; Rolan et al in 1994). Finally in one case the presence of LCT solutions didn't enhance the absorption of the drug compared with the tablets (Angelluci et al 1976). Despite the fact that the results seem to be case specific, there are indications that LCT oil solutions perform better compared with MCT solutions. However it is rather difficult to draw conclusions because of the limited number of studies and the fact that there are no clear indications of which characteristics of a compound that are determining whether LCT or MCT oils give better bioavailability.

Emulsions

Another approach to increase the bioavailability of poorly water soluble drugs is to formulate them in lipid emulsions.

Bioavailability studies in humans were carried out using o/w-emulsions, aqueous suspensions and commercial tablets (Bates and Sequeira 1975) of griseofulvin which is an antifungal antibiotic and it is commonly used in the treatment of dermatophyte infections in humans. Four dosage

forms containing micronized griseofulvin were evaluated by administration to humans. The corn o/w emulsion and aqueous suspension both contained 300 mg of polysorbate 60 in emulsion and 500 mg of suspended griseofulvin per 30 g of formulation. Two different commercial tablets contained 500 mg of micronized griseofulvin. The four dosage forms were administered in a random cross-over fashion to five fasting subjects in the form of 30 g of the corn oil emulsion, 30 g aqueous suspension and the tablets and the drug absorption was monitored from urinary excretion data for the major metabolite (6-desmethylgriseofulvin). Administration of o/w emulsion resulted in a 3–4 fold increase approximately, in the maximum excretion rate (mg/hr) of total 6-desmethylgriseofulvin. The bioavailability from the o/w emulsion increased 2 times compared with the other dosage forms. Such behavior attributed to the presence of linoleic acid contained in the lipolysis products which may inhibit the GI motility and stimulate the gallbladder evacuation.

In a follow up study the effect of different amounts of lipid emulsion was investigated (Bates et al 1977). However the total 6-desmethylgriseofulvin excretion rate profiles were quite similar after administration of the ultramicrosize griseofulvin tablets and 5, 10, 15, and 30 g of o/w emulsion used in the that study.

Lipid and surfactant based systems have been used for coenzyme Q10 (ubiquinone) which is a lipid soluble antioxidant, suggested to have a positive influence in congestive cardiac failure. The absorption of coenzyme Q10 was followed upon administration in different lipid dosage forms (Weis et al 1994). The tested formulations included 100 mg coenzyme Q10 i) suspended in soy bean oil (400 mg) administered in a soft gelatine capsule ii) in a mixture of polysorbate 80 (20 mg), phosphatidylcholine (100 mg) and soybean oil (280 mg) and iii) in a mixture of polysorbate 80 (20 mg) and soy bean oil (380 mg). The suspension in pure soy bean oil increased the bioavailability of coenzyme Q10 significantly compared with the lipid mixtures that included surfactant systems. It was suggested, that possibly the formation of mixed micelles containing polysorbate 80 phosphatidylcholine and soyabean oil for formulation ii) and micelles from polysorbate 80 and soyabean oil for formulation iii), in the intestine might induce a decrease of the drug solubilization with the bile salts.

Recently the effect of an organized lipid matrix emulsion containing lyso-phosphatidylcholine, monoglycerides, and fatty acids (ratio 1:4:2) was given along with a traditional capsule formulation of retinyl palmitate (Lepage et al 2002). The lipid matrix emulsion yields

a single melting point (64 °C) which is much lower than the melting point of its individual components. It produces an eutectic matrix due to the interaction of monoglycerides with the lyso-phosphatidylcholine. The absorption in cystic fibrosis adolescent patients, who have an impaired pancreatic function, was compared with that upon administration of the same dose of retinyl palmitate given along with a conventional isocaloric TG based nutritional supplement. It was concluded that the AUC (over baseline) for the organized lipid matrix was 10-fold higher compared with the TG based formula. The organized lipid matrix formula needed no hydrolysis before absorption, and could lead to a less variable absorption. This organized lipid matrix emulsion has also been tested in human subjects with the compound 4-Hydroxyphenyl-retinamide (4-HPR) a retinoid compound with antileukemic and proapoptotic activity in acute lymphoblastic leukemia. The bioavailability of a 4-HPR oral capsule formulation resulted in poor patient compliance at higher doses (due to the large number of capsules) in phase I trials of high-dose oral 4-HPR and high inter-patient variations. Using a lipid matrix emulsion it was possible to reduce the dose to 20% of the traditional formulation and still obtain equivalent peak plasma concentrations (Avanti® Polar Lipids, Inc. 2006).

Dry emulsions consisting of redispersed freeze-dried drug-milk formulations have been evaluated using reconstitution in vitro to obtain an o/w emulsion before administration for dicumarol (an anticoagulant) (Macheras and Reppas 1986a) and nitrofurantoin (a bacteriocidal) (Macheras and Reppas 1986b) respectively. Capsules containing the pure drugs were used as controls in both cases. Analysis of urine data revealed superiority of the nitrofurantoin-milk formulations regenerated with 200 and 400 ml of milk over the corresponding capsule formulations in the rates and extents of nitrofurantoin excretion. Determination of the plasma dicumarol levels indicated superiority of the dicumarol-milk formulation. Statistically significant differences were found between area under the curve, maximum plasma concentration, and apparent elimination rates.

The introduction of the organized lipid matrix emulsion which can be used with further hydrolysis is an interesting approach. The main advantage of this formulation is that no hydrolysis is needed which can result in less variations in absorption. However it is rather difficult to draw conclusions for the applicability of this formulation since the data from the literature are quite limited at the moment. Furthermore

the potential of the drug-milk freeze-dried formulations for the enhancement of the bioavailability of sparingly water soluble drugs are not yet elucidated.

Self emulsifying drug delivery systems

Self (micro) emulsifying drug delivery systems have attracted a lot of attention lately. Their thermotropic stability and the high drug loading efficiency make them a promising system for poorly water soluble drugs giving particles with small size.

Julianto and colleagues (2000) demonstrated that a SEDDS increased the bioavailability of α -tocopherol approximately 210%–410% compared with a commercially available soybean oil solution. The antioxidant α -tocopherol, a lipid soluble vitamin (vitamin E), is a poorly aqueous soluble compound. Traditionally α -tocopherol has been formulated as a capsule containing a simple oil solution. Both the SEDDS and the commercial α -tocopherol formulation contained 400 mg α -tocopherol. The SEDDS contained Tween 80: Span 80: Vitamin E (4:2:4) and the commercial formulation contained of α -tocopherol dissolved in soy bean oil. Both the $AUC_{0-\infty}$ and C_{max} values of the SEDDS were markedly higher than those of the oil solution, while the T_{max} was shorter, indicating a higher rate and extent of absorption. No physicochemical characterization was performed and it was not stated if the lipid amount were equal between the formulations.

In a recent study the bioavailabilities of three different lipid formulations of tocotrienols (Yap et al 2004) were investigated. Two self-emulsifying systems SEDDS, containing varying amounts of soybean oil, Tween 80 and Labrasol (C8/C10 polyglycolized glycerides from coconut oil) was analysed according to their self-emulsifying properties, droplet sizes and extent of lipolysis. The droplet size was 1.5 and 10.6 μ m respectively. Formulations containing 200 mg mixed tocotrienols administered in healthy adults as SEDDS or simple soybean oil solution stated that the SEDDS had markedly higher plasma levels and a faster onset of absorption compared with the oily solution. The droplet size difference between the two SEDDS did not affect the bioavailabilities. However, the total dosed lipid amounts between the three explored formulations ranged from 31.3–351.3 mg soybean oil thus making the interpretation of the bioavailabilities more complex.

The potent anti-HIV drug saquinavir has been enrolled in studies with human subjects in lipid based formulations. It has been formulated into SEDDS. The drug is available in

the market in both hard gelatine capsule (Invirase), containing saquinavir with lactose microcrystalline cellulose, povidone K30, sodium starch glycolate, talc and magnesium stearate and soft gelatin capsules (Fortovase), containing saquinavir in solutions in medium chain mono- and -diglycerides, povidone and dl- α tocopherol. In a recent study (Roche Laboratories, Inc 2004), a significant improvement to the bioavailability up to 331% of soft gelatine capsules compared with hard gelatine capsules. Ritonavir (Norvir) is available as in soft gelatin capsules with oleic acid, ethanol, polyoxyl 35 castor oil titanium dioxide, iron oxide and butylated hydroxytoluene (FDA 2006).

When healthy subjects were administered with saquinavir/ritonavir 1600 mg/100 mg, and no difference in AUC_{0-24} values between hard and soft gelatine capsules was observed, respectively (Cardiello et al 2003). The pharmacokinetics and the safety of a boosted saquinavir/ritonavir combination either with hard gelatin capsules or soft gelatin capsules was evaluated (Kurowski et al 2003). Comparable plasma exposures with saquinavir achieved when saquinavir boosted with ritonavir, accompanied by an improvement in gastrointestinal system disorders.

The turning point for development of the oral lipid and surfactant based formulations of poorly soluble drugs was the introduction to the market of cyclosporine A (CsA) in a lipid based formulation. CsA is an immunosuppressant used in chronic treatment of organ transplant receivers to suppress graft rejection and in the treatment of severe rheumatic arthritis and severe psoriasis.

The Sandimmune and Sandimmune Neoral formulations of CsA are perhaps the best known examples of a marketed lipid and surfactant based systems and the pharmacokinetic has been studied and reviewed extensively (Holt et al 1994; Ritschel 1996). Cyclosporine was introduced in 1981 in Europe in a self-emulsifying formulation (Sandimmune) containing Labrafil M 1944 CS (polyoxyethylated oleic glycerides), olive oil and ethanol (Klyashchitsky and Owen 1998). This formulation disperses, when diluted with water, into a polydisperse oil-in-water macroemulsion. In 1994 a new self-microemulsifying formulation (Sandimmune Neoral, referred to as Neoral in the following) was introduced, which emulsifies spontaneously into a microemulsion with a particle size smaller than 100 nm. This formulation contains Cremophor RH40 (polyoxyl hydrogenated castor oil), corn oil glycerides, propylene glycol and ethanol (Klyashchitsky and Owen 1998).

Drewe and colleagues investigated the absorption of CsA (150 mg) from three experimental formulations using

Sandimmune as reference in fasting healthy volunteers. Two of the experimental formulations were microemulsions with respectively fast and slow in vitro release. The microemulsions were formulated using polyethylene glycol, hydrogenated castor oil, medium chain triglycerides and low molecular weight glycols. The last experimental formulation was a solid micellar solution with fast in vitro release composed of sucrose monolaurate and propylene glycol. The fast releasing microemulsion and the fast releasing solid micellar solution exhibited significant higher C_{max} and bioavailability (141% and 139% of Sandimmune, respectively). The slow releasing microemulsion was equivalent to Sandimmune with respect to C_{max} and bioavailability. The T_{max} of the tested formulations were in the same range (Drewe et al 1992).

Mueller and colleagues (1994a) investigated the dose linearity of CsA from Sandimmune and Neoral in healthy volunteers who had been fasting 12 h prior to and 4 h after administration finding that Neoral exhibited linear dose AUC relationship in the range of 200 to 800 mg in contrast to Sandimmune. The relative bioavailability of Neoral compared with Sandimmune was found to be in the range of 174–239% but dependent on the actual dose, with the highest dose resulting in the largest difference in bioavailability. It must be noted that the bioavailability for Neoral and a number of the generic products of CsA exhibited marked pharmacokinetic variations in different human subpopulations even though they had been found bioequivalent. This discussion is however beyond the scope of this article and has been reviewed recently elsewhere (Dunn et al 2001; Pollard et al 2001). Studies in fasted healthy volunteers comparing Sandimmune (300 mg CsA) and Neoral (180 mg CsA) it was found that Neoral exhibit shorter T_{max} and higher C_{max} and AUC (Mueller et al 1994a, 1994b; Kovarik et al 1994).

Recently an experimental CsA self-emulsifying galactolipid formulation has been compared with Neoral in fasting healthy volunteers (Odeberg et al 2003). The self-emulsifying galactolipid formulation forms an emulsion with mean particle size of 16–20 μm . The galactolipid formulations exhibited identical T_{lag} and T_{max} but slightly lower C_{max} (94%) and AUC (84%) when compared with Neoral (Odeberg et al 2003). In another study the bioavailability of a non-SMEDDS, which is semisolid opaque oily suspension that in aqueous solution forms particles with a mean diameter 200 nm, and a SMEDDS formulation of cyclosporine were tested (Postolache et al 2002). The results showed that the non-SMEDDS formulation was not bioequivalent with the SMEDDS formulation due to

a statistical significant lower absorption rate. The authors claimed that with this study it has been demonstrated that in vivo the non-SMEDDS capsules are not totally interchangeable compared with the SMEDDS capsules. In a different approach cyclosporine lipid nanoparticles (lipospheres) were developed (Bekerman et al 2004) and the effect of composition and particle size investigated. These lipospheres consisted of phospholipids, Span 80, Tween 80, Tricaprin, and Cremophor RH 40. Cyclosporin dispersions systems resulting in particle size of 25 up to 400 nm prepared. Oral bioavailability indicated a correlation between the AUC and C_{max} and the particle size of the dispersion showing the highest values for particles with 25 nm diameter. The clinical formulation of Neoral used as a reference. This study demonstrated a correlation between the particle size and the oral bioavailability of cyclosporine formulations. Furthermore the composition and possibly the surface properties of the lipid nanoparticles (lipospheres) could affect the oral bioavailability of cyclosporine.

The above clearly demonstrate that SEDDS can be a promising formulation approach for poorly water soluble drugs. The efficiency of these systems is rather case-specific depending on the composition of the formulation used each time.

Surfactant-based solutions

Cremophor is commonly used excipient in lipid-based formulations (Strickley 2004). Cremophor EL may inhibit PGP (p-glycoprotein), a relevant efflux pump which is expressed in high amount in gut, biliary tract and the blood brain barrier, and enzymes like CYP3A which may contribute to first pass metabolism.

Cremophor EL, is used as a vehicle for the solubilization of a wide variety of hydrophobic drugs, including anesthetics, photosensitizers, sedatives, immunosuppressive agents and anticancer drugs including the anticancer agent paclitaxel (Taxol).

The role of the surfactants Cremophor EL and Tween 80 and the absorption rate of paclitaxel was investigated by Malingre and colleagues (2001). In this study the 2 formulations were tested; 6 mg/ml paclitaxel in Cremophor EL: ethanol 1:1 v/v or in polysorbate 80: ethanol 1:1 v/v. The Polysorbate 80 formulation performed better than Cremophor EL formulation, both with regard to AUC and C_{max} . The results demonstrated that the presence of Cremophor EL limited the oral absorption of paclitaxel and they highlight the need of designing a better drug formulation in order to increase the oral absorption of paclitaxel.

In another approach (Martin-Facklam et al 2002) saquinavir in hard gelatine capsules (Invirase) was administered with increasing doses of Cremophor EL (up to 5 g) in volunteers by mixing the content of Invirase capsules with the appropriate amount of Cremophor EL and dispersing it into hard gelatine capsules. When 5 g Cremophor EL were administered the C_{max} of saquinavir increased from 8.8 ± 8.5 nmol/l for control (Invirase capsule) to 112.7 ± 74.7 nmol/l respectively. Increasing levels of Cremophor increased the AUC of saquinavir. It is suggested that Cremophor EL acts as a modulator of the absorption process by inhibiting intestinal PGP.

Barker and colleagues (2003) explored a 50% (w/w) solid dispersion of α -tocopherol in Gelucire 44/14 that comprises a mixture of pegylated fatty acid esters and glycerides. Compared with a commercial oil solution of α -tocopherol a two-fold increase in absorption was found after administration of the solid dispersion of α -tocopherol. The $AUC_{0-\infty}$ and C_{max} were considerably higher after administration of the solid dispersion compared with the commercial product. Additionally, the lag phase prior to absorption of α -tocopherol from the solid dispersion was markedly shorter than for the commercial oil solution. Interestingly, the absorption profile appeared to be bi-phasic with a second peak after 10 hours, presumably due to a 10 h post-dose meal. The fact that this second phase of the absorption of α -tocopherol occurred after the volunteers were fed (at 10 h post-dose) according to the authors was possible due to the mobilisation of the lipid-absorbing pathway in response to food.

The data with surfactant-based formulations are too limited to draw safe conclusions on and more studies are needed to clarify their role since many possible mechanisms are in play. New excipients like Gelucire and Labrasol can solubilize many drugs and will possibly be used more frequently in the future. They are similar to the Labrafil and they consist of mixtures of mono-, di- and triglycerides and mono- and di-fatty acid esters of polyethyleneglycol with fatty acid compositions. However how these excipients perform in vivo is a crucial parameter for their success.

Food effect

The food-drug interactions may depend on several factors such as the physical and chemical characteristics of the drug, the size and the composition of the meal or the time of drug intake in relation with the meal (Singh 1999).

A claimed advantage of certain lipid-based formulations is that the food effect on poorly soluble drug can be

diminished as it has been demonstrated by Mueller and colleagues (1994b).

The impact of a high-fat meal on the pharmacokinetics of CsA from Sandimmune and Neoral has been investigated in healthy volunteers. In a study comparing Sandimmune (300 mg CsA) and Neoral (180 mg CsA) it was found that food influences the pharmacokinetics of CsA from Sandimmune more profoundly than from Neoral. A significant prolongation of T_{lag} and T_{max} and an increase in AUC was found for Sandimmune (Mueller et al 1994b). For the Neoral formulation the T_{lag} and T_{max} were only slightly and insignificantly increased whereas both C_{max} and AUC were reduced though significantly (Mueller et al 1994b). In the fasted state the relative bioavailability of CsA from Neoral was slightly higher (114% of Sandimmune) compared with Sandimmune but when administered after a high-fat meal the bioavailability from Neoral was considerable lower (71.4% of Sandimmune) than from Sandimmune (Mueller et al 1994b). In another study in which healthy volunteers was administered 7.5 mg/kg CsA after a high-fat breakfast it was found that the bioavailability of Neoral was 109.3% compared with Sandimmune (Gonzalez-Llaven et al 1999). The difference in relative bioavailability of Neoral compared with Sandimmune in the two studies performed by Mueller and colleagues (1994b) and Gonzalez-Llaven and colleagues (1999), could be explained by the difference in dose. When correcting the relative bioavailability for the difference in dose under the assumption of linear pharmacokinetics in the study by Mueller et al the bioavailability of Neoral is 190% and 119% of Sandimmune in fasted and fed state respectively. This emphasizes the importance of administering equal doses when investigating the food effect on different formulations.

The concentration versus time profile for Sandimmune show, for a considerable number of individuals, two peaks in both fasting individuals (Mueller et al 1994a, 1994b; Kovarik et al 1994) and individuals fed a high-fat breakfast (Mueller et al 1994b). This could suggest that the absorption of CsA from the Sandimmune formulation was limited by gastric emptying or the food consumed 4 h after administration.

A lipid emulsion of danazol was dosed to human subjects and the pharmacokinetics was studied (Charman et al 1993). A commercial formulation as a powder (hard gelatin capsule) and a lipid emulsion formulation of danazol were tested in both fed and fasted states. The commercial formulation contained 100 mg danazol in a capsule and the lipid formulation contained 3.43 mg of danazol per gram of emulsion dose. The lipid phase of the emulsion consisted of glycerol

mono-oleate. In the fasted state the AUC for the lipid based formulation was 5 time higher than the powder formulation. However, no significant difference observed between the two formulations in the fed state.

Unfortunately the data from the literature are rather limited can not give a clear answer to the question, whether the lipid formulations are able to overcome food effect.

Summary

Oral route is the preferred for the administration of drugs. Since a lot of potential drug candidates are poorly soluble in water, resulting in poor and variable oral bioavailability, many approaches have been employed in order to produce formulations with a high and reproducible bioavailability. Development of lipid based formulations has recently attracted a lot of attention. Lipid based systems are defined as emulsions, microemulsion, SEDDS, micellar suspensions, and oil solutions. However, most proof of concept studies have been carried out using animal models. As is obvious from the above the number of clinical studies evaluating the benefits of lipid based formulations is rather limited. The clinical data described in this article indicates that the studies could be separated in two different time-periods, based on the design and the performed clinical trials. In the first period which covers the period from the early sixties up to late eighties the lipid formulations mainly consist of an oil solution of the drug or an oil macroemulsion. The physicochemical characterization at this stage is rather poor or nonexistent. But in almost all the cases the lipid formulations or emulsions performed better than the tablets.

The second period for lipid based formulations starts in the first half of the 1990s, with the entrance in the market of a SMEDDS (Neoral) of cyclosporine which produces a finely dispersed microemulsion when diluted in an aqueous phase emphasized the impact of the physicochemical properties of the lipid vehicles compared with formulations tested before.

A number of studies have reported higher bioavailability with decreased particle size when comparing self-emulsifying formulations (Drewe et al 1992; Kovarik et al 1994; Gao et al 1998; Odeberg et al 2003). However the formulations investigated in these studies made use of either different surfactant and lipid phases or different ratios between the surfactant and the lipid phase. This fact impedes a conclusion with regard to the optimal composition of SMEDDS, since it is well described that formulations with matching particle size but different lipid phases can exhibit different bioavailability values (Khoo et al 1998).

The choice of surfactants and lipids is a crucial factor for the in vivo fate of the formulation. The lipids play a significant role since they can increase the drug solubility in lumen, can change the physical (Aungst 2000) and the biochemical barrier function (Benet 2001) of the GI tract and they can stimulate the lymphatic transport (Porter and Charman 1997).

Furthermore the food-drug interactions are important to drug absorption. Concomitant food intake has been demonstrated to lead to an increase in drug bioavailability. However when a microemulsion was administered a reduced effect of food on bioavailability was noticed (Mueller et al 1994b).

The fact that only four drug products, Sandimmune® and Sandimmune Neoral® (cyclosporin A), Norvir® (ritonavir), and Fortovase® (saquinavir) are on the market in combination with the hydrophobic nature of many drug candidates implies that the studies with lipid based formulations have to be more intensive and more systematic.

Finally the stability of these formulations is a parameter that has been rather underestimated since there is lack of studies and data on this issue. However the success of the each formulation in perspectives of being released in the market as a commercial product is highly dependant on its stability in long term, therefore more studies are required.

In conclusion more human bioavailability studies are needed to elucidate the mechanisms of action of these formulations. In parallel the development of new formulations well characterized in terms of their physicochemical properties will offer new insights on their performance in vivo.

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