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European Journal of Pharmaceutics and Biopharmaceutics 67 (2007) 96–105
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

The effect of different lipid based formulations on the oral absorption of lipophilic drugs: The ability of *in vitro* lipolysis and consecutive *ex vivo* intestinal permeability data to predict *in vivo* bioavailability in rats

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Received 3 December 2006; accepted in revised form 24 January 2007
Available online 31 January 2007

Abstract

The purpose of this study was to investigate the impact of different lipid based formulations of lipophilic drugs on *in vitro* solubilization and intestinal *ex vivo* permeability processes. Thereafter, to evaluate the ability of these *in vitro* and *ex vivo* results to predict the corresponding *in vivo* oral bioavailability data. The dissolution of dexamethasone and griseofulvin in long (LCT), medium (MCT) and short (SCT) chain triglyceride formulations was tested in a dynamic *in vitro* lipolysis model. Following the completion of the lipolysis, the permeability through the gut wall was tested in an *ex vivo* side-by-side diffusion chamber model. The absolute oral bioavailability of the drugs from the tested formulations was investigated in rats. The dynamic *in vitro* lipolysis experiments indicated an equivalent performance of the different formulations for dexamethasone, and a performance rank order of MCT > LCT > SCT > H_2O for griseofulvin. In the subsequent *ex vivo* permeability studies, the SCT formulation caused enhanced permeation with doubled permeability coefficient for both drugs. The *in vivo* bioavailability of both drugs correlated well with the *in vitro* data, i.e., LCT = MCT = SCT for dexamethasone and MCT > LCT > SCT > H_2O for griseofulvin, despite the significant augmented intestinal permeability produced by the SCT formulation. In conclusion, the *in vitro* lipolysis model was found to be useful in the intelligent optimization of oral lipid formulations for lipophilic drugs, even in the case where the intestinal permeability is enhanced by the formulation. The SCT vehicle showed to be a potential permeability enhancer; however, for class 2 compounds, the permeability does not correlate with *in vivo* bioavailability.

Keywords: Lipophilic drugs; Lipid based formulations; Bioavailability; Intestinal absorption; *In vitro* lipolysis; *Ex vivo* Ussing chamber; Intestinal permeability

1. Introduction

As a consequence of modern drug discovery techniques (i.e., advances in *in vitro* screening methods, the introduction of combinatorial chemistry), the number of poorly water-soluble drug candidates is constantly increasing. To date, more than 40% of new active chemical entities are lipophilic and exhibit poor water solubility [1]. These molecules suffer from low oral bioavailability, and thus fail to proceed to the advanced stages of research and development, despite their favorable pharmacological activity [2]. The most popular approach to improve the oral bioavailability of these molecules is the utilization of lipid based formulations [3–5].

The ability of lipid vehicles (either in the pharmaceutical formulation or in food) to enhance the absorption of lipophilic drugs has been well known for many years. The mechanisms behind this augmented bioavailability include

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doi:10.1016/j.ejpb.2007.01.017

enhanced dissolution and solubilization of the co-administered lipophilic drug by stimulation of biliary and pancreatic secretions, prolongation of gastrointestinal tract (GIT) residence time, stimulation of lymphatic transport, increased intestinal wall permeability and reduced metabolism and efflux activity [6,7].

Lipid-based formulations offer a large variety of optional systems. They can be made as solutions, suspensions, emulsions, self-emulsifying systems and microemulsions. Moreover, it is possible to form blends that are composed of several excipients: they can be pure triglyceride (TG) oils or blends of different TG, diglyceride (DG) and monoglyceride (MG). In addition, different types of surfactants (lipophilic and hydrophilic) can be added, as well as hydrophilic co-solvents. Lack of enhanced absorption when one of the above key formulations is tested does not necessarily indicate the effectiveness of alternative lipid-based formulations, and their suitability has to be examined. Unfortunately, the development strategies in the area of lipid formulations are mostly empirical, demand a large number of animal experiments, and consume time and money.

In order to aid in the suitable selection of the lipidic vehicle composition and rationalize the formulation design for drug candidate, different *in vitro* lipolysis methods have been suggested by several groups [8–11]. These methods were designed to mimic the GIT environment and reflect the intestinal conditions in terms of maintaining constant pH, the presence of lipase/co-lipase, and bile salts (BS) and phospholipid (PL) concentration. These models have also been shown to simulate other dynamic changes, including the formation of mixed micellar species that are generated throughout the interaction of the vehicle with the GIT environment [12]. A key goal in the development of these *in vitro* models is correlating the *in vitro* information of various drug-formulations to the *in vivo* drug profile. A model that reliably correlates *in vitro* and *in vivo* data shortens the drug development period, economizes resources and leads to improved product quality. A few studies have evaluated the *in vitro–in vivo* correlation (IVIVC) of the lipolysis model [13–16]; however, additional work is needed in order to verify the ability of this model to predict actual oral bioavailability of certain drug-vehicle combinations. Furthermore, data are needed on the potential influence of the vehicle on the permeability of the co-administered drug molecules through the gut wall. This issue does not take into account the *in vitro* lipolysis method.

The aim of the present study was to examine the impact of different lipid based formulations of two poorly water soluble, lipophilic drugs, on the two major barriers that a lipophilic drug has to traverse along the intestinal absorption cascade: the solubilization process using a dynamic *in vitro* lipolysis model, and the intestinal permeability process utilizing the *ex vivo* side-by-side diffusion chamber model. Subsequently, we compared these *in vitro* and *ex vivo* results with *in vivo* oral bioavailability data obtained for these drug-vehicle combinations. The *ex vivo* permeation experiment was performed immediately following the completion of the *in vitro* lipolysis study, utilizing the same medium, in order to maximize the simulation of the physiological GIT absorption process. For the first time, this data set will enable the evaluation of the influences of lipid based formulations on the consecutive barriers facing the absorption of the lipophilic drug from the GIT, i.e., solubilization and permeation, and to examine the ability of these *in vitro* and *ex vivo* models to predict the actual *in vivo* performance of the tested formulations.

For these investigations we selected two model lipophilic drugs which share relatively similar $\log P$ values, but different water solubility characteristics: the steroid dexamethasone which has a water solubility of 100 μg/ml ($\log P = 1.8$) [17–19] and the antifungal drug griseofulvin which is 20-fold less soluble in water (5 μg/ml; $\log P$ value of 1.8–2.1 is reported in the literature) [18,20,21]. These two model drugs are classified as class 2 compounds by the biopharmaceutical classification system (BCS) [22], and hence represent the majority of lipophilic drugs. Three lipidic vehicles were tested for each molecule: peanut oil (C_{18}, long chain triglycerides (LCT)), Captex 355 (C_{10}, medium chain triglycerides (MCT)) and triacetin (C_2, short chain triglycerides (SCT)) based formulations.

2. Materials and methods

2.1. Materials

Dexamethasone, testosterone, peanut oil (LCT), triacetin (SCT), taurocholic acid, pancreatin (8 × USP), L-α-phosphatidylcholine, tris maleate and calcium chloride were purchased from Sigma Chemical Co. (St. Louis, MO). Micronized griseofulvin BP was generously supplied by Teva Pharmaceutics (Jerusalem, Israel). Captex 355, triglycerides of caprylic/capric acid (MCT), was generously supplied by Abitec Corporation Co. (Janesville, WI). Normal saline solution was obtained from Teva Medical (Ashdod, Israel). Ethanol, methanol, acetonitrile, water, *n*-hexane potassium chloride, magnesium chloride and sodium chloride (J.T. Baker, Deventer, Holland) were HPLC grade. All other chemicals were of analytical reagent grade.

2.2. In vitro dynamic lipolysis experiments

The procedure for the dynamic *in vitro* lipolysis experiment was designed to achieve maximum pseudo-physiological conditions, as previously reported [15]. The experimental medium, which was comprised of 35.5 ml of digestion buffer (50 mM tris maleate, 150 mM NaCl, 5 mM $CaCl_2$, pH 6.8) containing 5 mM taurocholic acid and 1.25 mM L-α-phosphatidylcholine (conditions mimicking fasted state GIT), was continuously stirred (100 rpm) and maintained at 37 °C. One gram of the tested formulation (a suspension containing 5 mg of the drug, stirred and gently heated (37 °C), freshly prepared 30 min before each

experiment) was then dispersed in the medium and stirred for 15 min. Fresh pancreatin extract was prepared by adding 1 g of porcine pancreatin powder to 5 ml digestion buffer, stirring for 15 min followed by centrifugation, as previously described in the literature [23]. About 3.5 ml of the pancreatin extract (1000 IU/ml) was inserted into the medium and initiated the enzymatic digestion of the formulation. In order to achieve maximum pseudo-physiological conditions a pH-titrator unit was used (DL-50 Graphix, Mettler Toledo Inc., Columbus, OH) to maintain the pH at 6.8 throughout the experiment. This is important since during the lipolysis process of triglycerides, free fatty acids are liberated and consequently the pH decreases. The experiment was continued for 30 min, in which time the enzymatic digestion process was completed, as indicated by the completion of the pH titration.

In order to achieve maximal simulation of the physiological absorption process, 5 ml of the medium was transferred to the consecutive permeation study in the side-by-side Ussing diffusion chambers, immediately following the completion of the lipolysis process. The rest of the medium was ultracentrifuged (L8-55 Ultracentrifuge, SW-41 rotor, Beckman Co., Palo Alto, CA) at 40 K rpm, 37 °C, for 90 min, and separation into three phases was obtained: an aqueous phase (containing bile salts, fatty acids and MG), a lipid phase (containing undigested TG and DG) and sediment (containing undissolved fatty acids). Each of the phases was analyzed for drug content. Following oral administration, dissolution of the drug molecule in the intestinal milieu is a prerequisite for the absorption process. Hence, drug molecules solubilized in the aqueous phase of the lipolysis medium are thought to be available for absorption, in contrast to drug in the sediment which will not be available for absorption in *in vivo* conditions.

In order to outline the propagation of the lipolysis process and its dynamic influences on the solubilization of the co-administered drug, a similar procedure was performed with additional aliquots taken at 5, 10, 15 and 20 min following the initiation of the lipolysis process (i.e., the insertion of the pancreatin extract). Aliquots were immediately frozen (−80 °C, Revco, Thermo Electron Corporation, Asheville NC) in order to stop the enzymatic activity. Continued procedures were as described above.

2.3. Ex vivo permeation experiments

Permeability experiments were performed in a modified Ussing chamber system (Physiological Instruments Inc., San Diego, CA) [24] by a method described before [25] with some modifications. In this model, small section of the intestine is clamped between two chambers filled with buffer. One chamber contains drug molecules (the donor cell) and the other one does not (the receiver cell), allowing permeation experiments through the mounted tissue. Male Wistar rats (Harlan, Israel) of 275–300 g in weight were used in these studies. Following a midline incision, 25 cm of small intestine was removed and placed in ice-cold Ringer bicarbonate buffer (NaCl 6.54 g, KCl 0.37 g, $CaCl_2 \cdot 2H_2O$ 0.18 g, $MgCl_2 \cdot 6H_2O$ 0.24 g, $NaHCO_3$ 2.1 g, Na_2HPO_4 0.23 g, NaH_2PO_4 0.05 g in 1000 ml). All buffer solutions were freshly prepared and equilibrated to pH 7.4, and osmolality 290 mOsm. The jejunal portion of the small intestine (10–15 cm distal to the pylorus) was used. Peyer patches could be easily identified visually, and sections containing them were not used in these studies. The individual segments were obtained and underlying muscularis was removed from the serosal side of the tissue before mounting. The exposed tissue surface area was 0.5 cm^2 and fluid volume in each half-cell was 3 ml. The system was preheated to 37 °C.

The medium of the *in vitro* lipolysis experiment, immediately following the completion of the lipolysis process, was added into the mucosal side of the Ussing chambers. This medium contained the digestion buffer, the drug molecules in the different states, i.e., dissolved in the medium or suspended solid, and the lipolytic products liberated from the tested lipidic vehicle following the lipolysis process. Modified Ringer buffer was added to the serosal side (serosal-modified Ringer buffer contained 8 mM D-glucose). The tissue oxygenation and the solution mixing were performed by bubbling with 95% O_2–5% CO_2. The system was equilibrated for 30 min and followed thereafter by permeability experiments which continued for 150 min. Samples were withdrawn from the serosal side at predetermined times and the sampled volume was replaced by blank (non-drug containing) medium to maintain sink conditions. The integrity of epithelial tissue was monitored by measuring the transepithelial electrical resistance (TEER) throughout the experiment (20 min intervals during 5 s). Viability was assessed by applying 1 μM forskolin at the end point (180 min) and an increase or decrease of above 5% in I_{sc} (the current that reflects the flux of ions from one cell to another) was observed. Any tissue with values $<30\ \Omega\ cm^2$ was discarded before the start of the experiment. Generally, TEER values were $90 \pm 20\ \Omega\ cm^2$ and remained steady throughout the experiment. These values represent a good viability and integrity of the tissue throughout the experiment [26,27]. Similar TEER values were obtained in control groups of blank vehicle as well.

Permeability coefficient (P_{app}): the P_{app} for each compound and formulation was calculated from the linear plot of drug accumulated in the serosal side versus time, using the following equation:

$$P_{app} = \frac{1}{C_0 A} \times \frac{dQ}{dt} \quad (1)$$

where dQ/dt is a steady-state appearance rate of the drug on the receiver (serosal) side, C_0 is the initial concentration of the drug on the donor (mucosal) side, and A is the exposed tissue surface area (0.5 cm^2). Linear regression was carried out to obtain the steady-state appearance rate of the drug on the serosal side ($R^2 > 0.99$ in all experimental groups).

2.4. Bioavailability studies in rats

All surgical and experimental procedures were reviewed and approved by the Animal Experimentation Ethics Committee of the Hebrew University Hadassah Medical School Jerusalem. Male Wistar rats (Harlan, Israel), 275–300 g in weight, were used for all surgical procedures.

Animals were anesthetized for the period of surgery by intra-peritoneal injection of 1 ml/kg of ketamine–xylazine solution (9%:1%, respectively) and placed on a heated surface maintained at 37 °C (Harvard Apparatus Inc., Holliston, MA). An indwelling cannula was placed in the right jugular vein of each animal for systemic blood sampling, by a method described before [28]. The cannula was tunneled beneath the skin and exteriorized at the dorsal part of the neck. After completion of the surgical procedure, the animals were transferred to metabolic cages to recover overnight (12–18 h). During this recovery period and throughout the experiment, food, but not water, was deprived. Animals were randomly assigned to the different experimental groups.

In order to study the absorption of dexamethasone, the steroid (5 mg/kg), suspended in either peanut oil (LCT), Captex 355 (MCT) or triacetin (SCT) (5 mg/ml, w/v, freshly prepared 30 min before each experiment), was administered to the animals by oral gavage ($n = 5$ rats in each group). Systemic blood samples (0.25 ml) were taken at 5 min pre-dose, 0.5, 1, 2, 3, 4 and 6 h post-dose. To prevent dehydration, equal volumes of normal saline solution were administered to the rats following each withdrawal of blood sample.

The same procedure was used for the griseofulvin oral absorption study. The antifungal drug (50 mg/kg), suspended in either LCT, MCT or SCT (50 mg/ml), was administered to the animals by oral gavage. Blood samples were taken at 5 min pre-dose, 1, 2, 4, 6, 8, 12, 18 and 24 h post-dose.

In order to determine absolute bioavailability of dexamethasone and griseofulvin, two additional groups of animals ($n = 4$) received intravenous injection (1 ml/kg) of dexamethasone (5 mg/kg) or griseofulvin (25 mg/kg), dissolved in ethanol:propylene glycol:saline (3:3:4, respectively).

2.5. Analytical methods

The amount of dexamethasone and griseofulvin in plasma and in the *in vitro* digestion medium was determined using a high performance liquid chromatography (HPLC) system (Waters 2695 Separation Module) with a photodiode array UV detector (Waters 2996). Plasma or medium aliquots of 100 μl were mixed with 200 μl of ethanol containing the internal standard (testosterone, 1 μg/ml) and vortex-mixed for 1 min. Two milliliters of *n*-hexane was added, followed by 1 min vortex-mixing. After centrifugation at 1500*g* for 10 min, the organic layer was transferred, evaporated (Vacuum Evaporation System, Labconco, Kansas City, MO) and reconstituted in 50 μl of ethanol. Ten microliters of the resulted solution was injected into the HPLC system. The HPLC conditions were as follows: XTerra, RP_{18}, 3.5 μm, 4.6 × 100 mm column (Waters Co., Milford, MA), an isocratic mobile phase, acetonitrile:water (70:30 v/v), flow at a rate of 1 ml/min in room temperature. Dexamethasone and griseofulvin were detected at 242 and 294 nm, respectively.

Separate standard curves were used for plasma and the *in vitro* digestion medium samples ($R^2 > 0.999$). The minimum quantifiable concentrations for dexamethasone and for griseofulvin were 20 and 10 ng/ml, respectively. The inter- and intra-day coefficients of variation were <1.0 and 0.5%, respectively.

2.6. Pharmacokinetic analysis

Plasma concentrations versus time data obtained for dexamethasone and griseofulvin in individual rats were analyzed by means of the noncompartmental analysis model using WinNonlin® Professional software version 4.0.1. The absolute bioavailability of the drugs was calculated from the ratio of the AUC values normalized by dose after oral and intravenous administration.

2.7. Statistical analysis

All values are expressed as means ± standard deviation (SD). To determine statistically significant differences among the experimental groups, the non-parametric Kruskal–Wallis test was used for multiple comparisons, and the two-tailed non-parametric Mann–Whitney *U* test for two-group comparison when appropriate. A p value of less than 0.05 was termed significant.

3. Results

3.1. In vitro dynamic lipolysis model results

The distribution and solubilization pattern of dexamethasone and griseofulvin across the different phases of the digestion lipolysis medium resulting from the LCT, MCT and SCT formulations are presented in Figs. 1 and 2, respectively, and summarized in Table 1.

For dexamethasone, the data demonstrate that the different lipid based formulations do not differ from one another, and all of the three chain lengths that were investigated enabled the solubilization of high amounts of the drug in the aqueous phase. Since the drug molecules that solubilized in the aqueous phase of the lipolysis medium are thought to be available for absorption, similar performance rank (i.e., LCT = MCT = SCT) is expected for these dexamethasone formulations in *in vivo* conditions by this dynamic *in vitro* lipolysis model.

On the other hand, the data for griseofulvin show significant differences between the three investigated lipidic vehicles, and that formulating with MCT, rather than LCT or

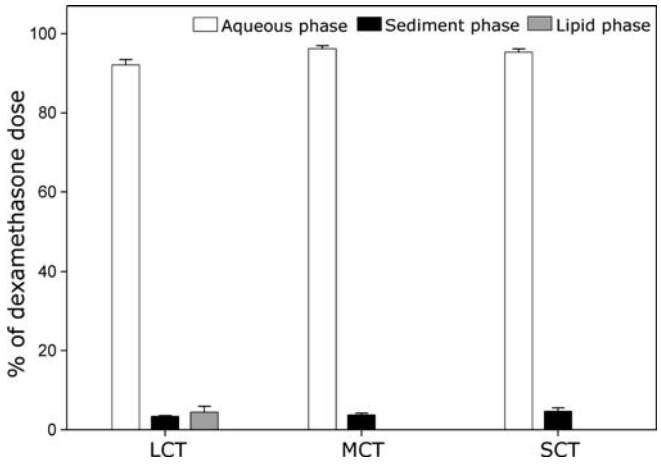

Fig. 1. Distribution of the dexamethasone molecules across the aqueous phase (□), sediment (■) and lipid phase (▩) of the dynamic *in vitro* lipolysis medium following LCT, MCT or SCT lipid based formulation. Data presented as means ± SD, $n = 6$ for each formulation.

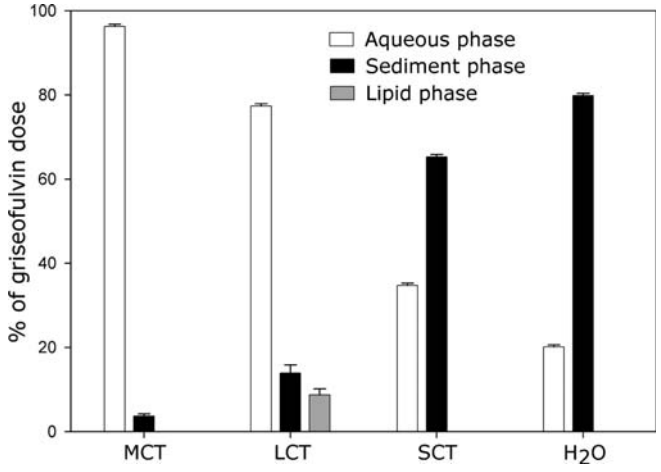

Fig. 2. Distribution of the griseofulvin molecules across the aqueous phase (□), sediment (■) and lipid phase (▩) of the dynamic *in vitro* lipolysis medium following LCT, MCT and SCT lipid based formulation, or aqueous suspension. Data presented as means ± SD, $n = 6$ for each formulation.

SCT, leads to extremely high amounts of solubilized drug in the aqueous phase (>95% of the drug dose). The LCT formulation resulted in a lesser amount of the drug molecules in the aqueous phase in comparison to the MCT, while the SCT vehicle provided the poorest results among the three oily formulations. Since significant differences were obtained in the griseofulvin experiments, an additional control group of aqueous suspension was tested. All three lipid based formulations proved to be advantageous over the control of an aqueous suspension.

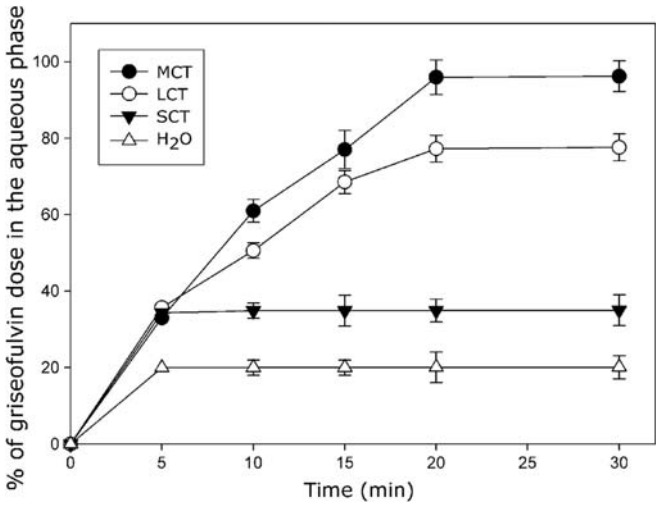

Fig. 3. Propagation of the lipolysis process and its dynamic influences on griseofulvin solubilization in the aqueous phase following LCT (○), MCT (●) and SCT (▼) lipid based formulation, or aqueous suspension (△) ($n = 4$ for each formulation).

The propagation of the lipolysis process throughout time and its influences on the griseofulvin dissolution in the aqueous phase are shown in Fig. 3. It can be seen that while the LCT and the MCT vehicles were influenced profoundly by the lipolysis process, the SCT and the aqueous suspension were not affected by the lipolysis, as griseofulvin dissolution reached plateau very fast (less than 5 min, while lipolysis was still carried out) in the case of the last two. As denoted above, drug molecules that precipitated during the lipolysis process in this biorelevant medium are not expected to be available for absorption in *in vivo* conditions. Hence, the performance rank order of these vehicles in *in vivo* conditions by this dynamic *in vitro* lipolysis model is MCT > LCT > SCT > H_2O.

Table 1
Distribution (% of dose) of the dexamethasone and the griseofulvin molecules across the aqueous phase, sediment and lipid phase of the dynamic *in vitro* lipolysis medium following LCT, MCT or SCT lipid based formulation, and following aqueous suspension where investigated

The formulation	Griseofulvin (% of dose)			Dexamethasone (% of dose)		
	Aqueous phase	Sediment	Lipid phase	Aqueous phase	Sediment	Lipid phase
LCT	77.6 ± 0.5	13.7 ± 1.9	8.7 ± 1.4	92.1 ± 1.3	4.5 ± 0.16	3.4 ± 1.4
MCT	96.2 ± 0.5	3.8 ± 0.5	No lipid phase	96.2 ± 0.7	3.8 ± 0.4	No lipid phase
SCT	35 ± 0.4	65 ± 0.5	No lipid phase	95.3 ± 0.8	4.7 ± 0.8	No lipid phase
H_2O	20.1 ± 0.5	79.9 ± 0.5	No lipid phase	ND		

Data presented as means ± SD, $n = 6$ for each formulation. ND, not determined.

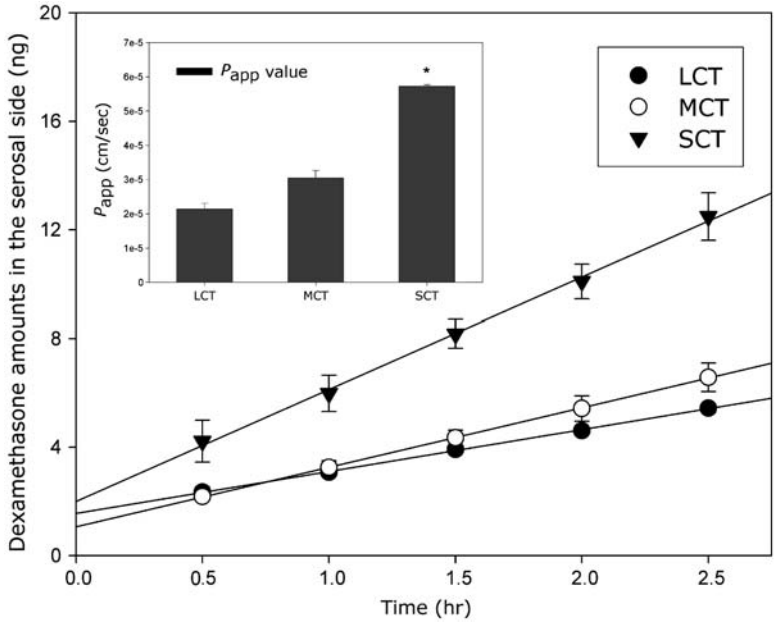

Fig. 4. Dexamethasone amount accumulated in the serosal cell versus time of the ex vivo side-by-side Ussing chamber model, resulting from the LCT (●), MCT (○) and the SCT (▼) formulations (mean ± SD, $n = 6$). The corresponding permeability coefficients (P_{app}) obtained presented in columns.

3.2. Ex vivo permeation experiments

The linear plot of drug accumulated in the serosal cell versus time of the side-by-side Ussing chamber model for dexamethasone and griseofulvin resulting from the LCT, MCT and SCT formulations, and the corresponding permeability coefficients obtained, are presented in Figs. 4 and 5, respectively, and summarized in Table 2. The data demonstrate, for both dexamethasone and griseofulvin, that formulating with SCT, rather than LCT or MCT, leads to enhanced permeation of the drug through the gut wall, with doubled permeability coefficient (P_{app}) value in comparison to the other two oils.

3.3. In vivo absorption of dexamethasone and griseofulvin in rats

The plasma concentration time profiles for dexamethasone and griseofulvin following oral administration of the different formulations are shown in Figs. 6 and 7,

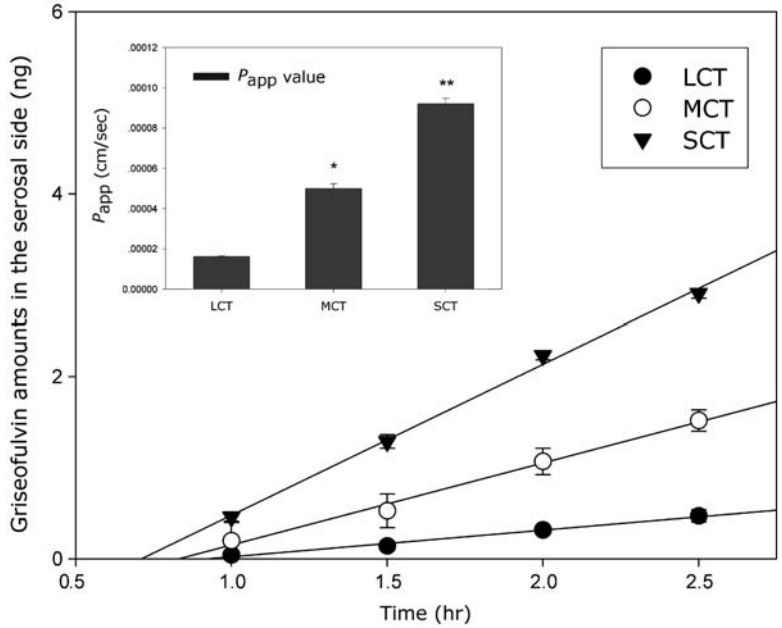

Fig. 5. Griseofulvin amount accumulated in the serosal cell versus time of the *ex vivo* side-by-side Ussing chamber model, resulting from the LCT (●), MCT (○) and the SCT (▼) formulations (mean ± SD, $n = 6$). The corresponding permeability coefficients (P_{app}) obtained presented in columns.

Table 2
Dexamethasone and griseofulvin permeation coefficient (P_{app}) values (cm/s) obtained in the *ex vivo* Ussing chamber model following LCT, MCT or SCT lipid based formulation

TG type in the formulation	Griseofulvin P_{app} values (cm/sec)	Dexamethasone P_{app} values (cm/sec)
LCT	1.6E−05 ± 2.5E−07	2.1E−05 ± 1.6E−06
MCT	5.0E−05 ± 2.3E−06	3.0E−05 ± 2.1E−06
SCT	9.2E−05 ± 2.6E−06	5.7E−05 ± 3.5E−07

Data presented as means ± SD, $n = 6$ for each formulation.

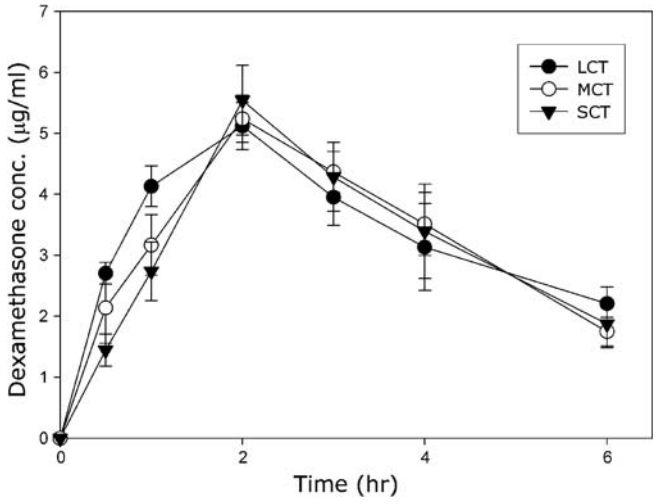

Fig. 6. Plasma dexamethasone concentration–time profiles (mean ± SD), following oral administration of 5 mg/kg dexamethasone LCT (●), MCT (○) or SCT (▼) based formulation ($n = 5$ for each formulation).

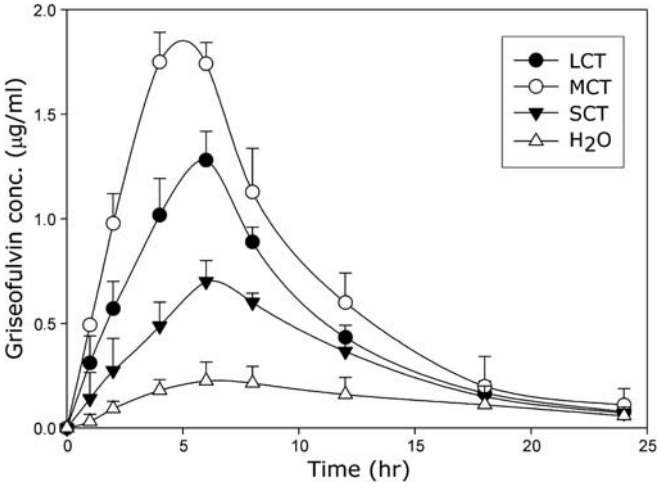

Fig. 7. Plasma griseofulvin concentration–time profiles (mean ± SD), following oral administration of 50 mg/kg griseofulvin LCT (●), MCT (○) and SCT (▼) lipid based formulation, or aqueous suspension (△) ($n = 5$ for each formulation).

respectively. The corresponding pharmacokinetic parameters obtained in these *in vivo* experiments for both dexamethasone and griseofulvin are listed in Table 3.

The data show that the absorption of dexamethasone from the different lipid based formulations does not differ from one another, and all of the three chain lengths that were investigated yielded equivalent bioavailability. This trend, i.e., LCT = MCT = SCT, is in correlation with the data obtained from the *in vitro* dynamic lipolysis model.

The bioavailability of griseofulvin from the MCT based formulation was greater in comparison to the LCT vehicle, and the SCT formulation resulted in the poorest bioavailability amongst the three tested lipidic vehicles. All three lipid based formulations proved to be advantageous over the aqueous suspension. These results correlate well with the data obtained from the dynamic *in vitro* lipolysis experiment, and the expected performance rank order of MCT > LCT > SCT > H_2O proved to be correct in *in vivo* conditions. Moreover, the correlation between the percent of griseofulvin dose that was solubilized in the aqueous phase of the *in vitro* lipolysis medium and the *in vivo* AUC values following oral administration of the corresponding vehicles is presented in Fig. 8. It can be seen that a strong correlation ($R^2 > 0.98$) was obtained between these parameters. It should be noted that this correlation only reflects the state for the four tested formulations, and may not be valid for other different TG blends.

4. Discussion

In vitro models to help predict the fate of a poorly water soluble drug in a lipid based delivery system upon its dilution and digestion in the GI milieu are highly needed, and have received increasing attention in recent years [9,10,29–31]. In this study, we utilized a dynamic *in vitro* lipolysis model that enables a very good reflection of the dynamic formation of mixed micellar species throughout the lipolysis process, and which simulates intestinal fast state conditions in terms of maintaining constant pH, the presence of lipase/co-lipase and having proper BS and PL concentrations. However, further work is needed in order to assess the advantages and limitations of this *in vitro* model in predicting the *in vivo* performance of different drug-vehicle combinations. In particular, there is an interesting question about the impact of the different lipid based formulations on the permeation of the co-administered lipophilic drug through the gut wall, and the influence of this impact on the IVIVC. In the current investigation this question was examined, utilizing consecutive investigational models that maximize the simulation of the physiological GIT absorption process.

4.1. In vitro lipolysis studies

In this study, dexamethasone and griseofulvin were selected as model lipophilic, poorly water soluble compounds. It has been shown before that lipid based formulations can improve the oral bioavailability of griseofulvin [32,33]. The data presented in this paper clearly demonstrate that the nature of the lipid in the formulation of this

Table 3
Dexamethasone and griseofulvin pharmacokinetic parameters obtained following oral administration of LCT, MCT or SCT lipid based formulation, and following aqueous suspension where investigated

The formulation	Griseofulvin				Dexamethasone			
	AUC (h μg/ml)	k (h^{-1})	$t_{1/2}$ (h)	F (%)[a]	AUC (h μg/ml)	k (h^{-1})	$t_{1/2}$ (h)	F (%)[a]
LCT	12.3 ± 0.8	0.15 ± 0.01	4.5 ± 0.4	45.9 ± 6.7	29.1 ± 3	0.2 ± 0.03	3.4 ± 0.6	91.2 ± 15
MCT	17.6 ± 2.2	0.15 ± 0.02	4.4 ± 0.6	65.7 ± 10	27.6 ± 2.3	0.26 ± 0.02	2.5 ± 0.3	86.5 ± 12
SCT	8.2 ± 0.6	0.14 ± 0.01	5 ± 0.9	30.6 ± 4.7	26.5 ± 3.3	0.26 ± 0.03	2.6 ± 0.3	83 ± 16
H_2O	3.6 ± 0.4	0.13 ± 0.02	5.3 ± 1	13.4 ± 4.7	ND			

$n = 5$ rats in each group. Values presented as means ± SD. ND, not determined.
[a] Absolute bioavailability. AUC values following intravenous administration of 5 mg/kg dexamethasone and 25 mg/kg griseofulvin were 31.9 ± 2.7 (h μg/ml) and 13.4 ± 1.1 (h μg/ml), respectively.

antifungal drug directly influences the performance of the formulation in terms of enhanced drug dissolution and solubilization. On the other hand, the data show that the type of oil in the dexamethasone formulation does not affect these parameters. This finding is interesting in light of the relatively similar log P value of these two substances. This phenomenon may be attributed to the different water solubility properties of dexamethasone and griseofulvin. Although sharing a similar log P, dexamethasone is characterized by higher water solubility, and has a water solubility of 100 μg/ml [17]. This value represents a slightly soluble compound, yet not completely insoluble. In fact, this is the maximal water solubility a compound may have and still be defined as poorly water soluble. Hence, in the biorelevant medium of the lipolysis experiment, the presence of the BS and the PL was sufficient to solubilize the dexamethasone molecules in the aqueous phase to a high extent, regardless of the lipolytic products produced from the lipid of the formulation. Griseofulvin on the other hand is 20-fold less soluble in water (5 μg/ml) [20], and the free FA liberated from the lipid component of the formulation throughout the lipolysis process are crucial in order to enable the solubilization of this very poorly soluble drug in the aqueous phase. Hence, the diverse chain length of the administered lipids, which upon lipolysis produced different amounts and kinds of lipolytic products and colloidal phases, enabled a different degree of solubilization of the griseofulvin molecules in the aqueous phase. This point is further evident by the propagation of the lipolysis throughout the process, and its dynamic influences on griseofulvin solubilization (Fig. 3). It can be seen that aqueous suspension was not influenced by the lipolysis, due to absence of any lipidic component in the vehicle. The same trend was obtained in the case of SCT, probably due to the very small surface activity of its lipolytic products. Hence, in these two cases, griseofulvin dissolution reached its plateau within a few minutes, regardless of the lipolysis process. On the other hand, the LCT and MCT formulations, which upon lipolysis produce good surface active agents, showed a lipolysis dependent dissolution profile of griseofulvin in the medium. Kossena et al. have characterized the different elements produced during *in vitro* lipolysis of LCT and MCT lipids [11]. The authors reported that during the digestion of LCT lipid the main colloidal component that formed was mixed micelles, in contrast to the situation following MCT lipid digestion, in which the majority of colloidal component was vesicles. Vesicles appear to be quantitatively more important than mixed micelles in terms of drug solubilization. The data obtained in the present study also reflect this mechanism. Whereas following lipolysis of a MCT based griseofulvin formulation, more than 95% of the drug dose solubilized in the aqueous phase, lesser amount (77%) solubilized in the aqueous phase following LCT based griseofulvin formulation.

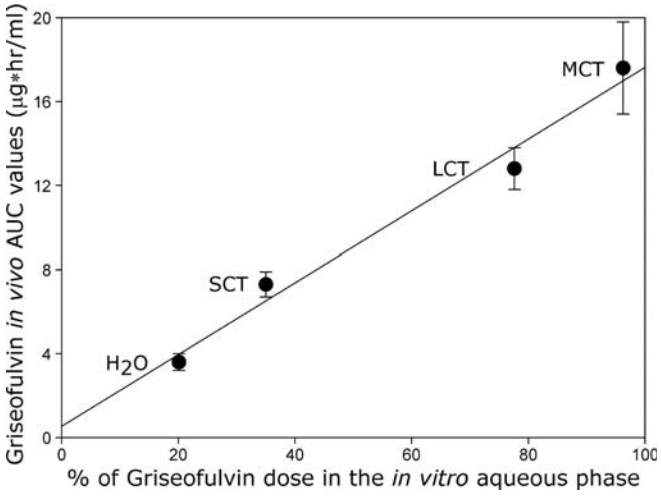

Fig. 8. Correlation between the percent of griseofulvin dose that solubilized in the aqueous phase of the *in vitro* lipolysis medium and the *in vivo* AUC values following oral administration of the corresponding vehicles ($R^2 > 0.98$).

A similar trend obtained in the *in vitro* lipolysis model was observed in the *in vivo* experiments as well. The absorption of dexamethasone from the three tested lipid based formulations was equivalent, whereas the magnitude of griseofulvin absorption was directly influenced from the nature of the oil component in the formulation. Kaukonen et al. have recently investigated the solubilization behavior of griseofulvin during *in vitro* lipolysis of LCT and MCT based suspensions [34]. The authors reported very similar results to the data presented in this paper. The propagation of the lipolysis process throughout time, the additional

SCT based formulation and aqueous suspension, as well as the *in vivo* evaluation of these drug-formulation combinations provided in this work, help to improve the understanding of the role of a lipid component in oral formulations.

4.2. Ex vivo permeation studies

A variety of lipids, e.g., medium chain fatty acid (decanoic acid or its derivatives), have been shown to change the physical barrier function of the gut wall, and hence, to enhance permeability. This membrane perturbation was proposed to be caused by the interaction between the enhancer and membrane proteins or lipids [35] resulting in an increase in the transcellular permeation. The data shown in this paper illustrate this phenomenon for both dexamethasone and griseofulvin. Moreover, the results of the *ex vivo* permeation studies pointing in that the lipolytic products of SCT, rather than MCT or LCT, have the greater ability to act as permeation enhancers amongst the three tested types of oils. While the mechanism of this effect is not completely clear it could be that the effect is contributed indirectly by the impact of the SCT lipid lipolytic products on the size of the formed vesicles, as well as possible effects on the enterocyte luminal membrane.

Nonetheless, no correlation was obtained between the outcomes of the *ex vivo* permeation model and the *in vivo* bioavailability data. While the *ex vivo* permeation experiments indicated that the SCT based formulation was expected to yield the highest *in vivo* absorption among the three tested formulations for both dexamethasone and griseofulvin, *in vivo* assessment following oral administration revealed that this is not the case, and the actual *in vivo* performance of the tested formulations was equivalent for dexamethasone (LCT = MCT = SCT), and a rank order of MCT > LCT > SCT > H_2O was observed for griseofulvin. This observation may be explained by the physicochemical properties of these two poorly water soluble drugs. Both dexamethasone and griseofulvin are classified as BSC class 2 compounds [36], i.e., poor solubility along with high permeability characteristics. Hence, the solubility of these drug molecules in the GIT milieu, rather than the permeation of the drug molecules through the gut wall, is the rate limiting step in the GI absorption process. In order to enhance bioavailability, it is important to identify the rate limiting step in the absorption process, and to counter the relevant barrier in each case. These data illustrate that for such molecules, i.e., BSC class 2 compounds, it is inefficient to enhance the permeation through the gut wall, and that the barrier that should be tackled in these cases is the dissolution and solubilization of the drug molecules in the GIT milieu.

4.3. In vitro–in vivo correlation (IVIVC)

The data presented in this paper clearly demonstrate that the nature of the lipid in the griseofulvin formulation directly influences the magnitude of griseofulvin absorption. The notable finding is that the *in vitro* lipolysis experiment can predict the *in vivo* performance of different vehicles. The strong positive correlation ($R^2 > 0.98$) obtained between the percent of griseofulvin dose solubilized in the aqueous phase of the *in vitro* lipolysis medium and the *in vivo* AUC values following oral administration of the corresponding vehicle (Fig. 8) highlights two key points. On the one hand it illustrates the ability of this dynamic *in vitro* lipolysis model to mimic the conditions of the *in vivo* dissolution process within the gut. On the other hand it clarifies that for this type of drug intestinal permeation studies cannot indicate the actual overall bioavailability *in vivo*.

A good IVIVC was obtained in the case of dexamethasone as well. In both the *in vitro* dynamic lipolysis model and in the *in vivo* bioavailability experiments, all three tested lipid based formulations demonstrated equivalent performance (LCT = MCT = SCT), and thus, the *in vitro* lipolysis model has been shown to be indicative for actual *in vivo* performance of different vehicles. Once again, the *ex vivo* permeation studies failed to predict the actual *in vivo* situation, and the increased *ex vivo* P_{app} value resulted from the SCT vehicle was not demonstrated *in vivo*.

It should be noted that neither dexamethasone nor griseofulvin has a component that is absorbed via the lymphatic system in their absorption process. We have previously demonstrated that for vitamin D_3, a fat soluble vitamin which has a significant lymphatic absorption [37], the *in vitro* lipolysis model is not suitable to predict the *in vivo* performance of different formulations [15]. Hence, for molecules which the lymphatic transport pathway has been shown to be a significant contributor for the overall bioavailability, care must be taken, and the *in vitro* lipolysis data have to be interpreted adequately.

5. Conclusions

In conclusion, the results presented in this paper show that valuable information can be obtained from the *in vitro* lipolysis model, leading to the intelligent selection of suitable lipid components for enhanced oral bioavailability of BCS class 2 compounds.

The present study illustrates the capability of different lipids to act as permeation enhancers, and that the lipolytic products of SCT have the greatest ability to act as permeation enhancers among the tested lipids. Yet for BCS class 2 compounds the impact of the different components of the formulation on the permeability of the drug molecules through the gut wall cannot indicate the actual *in vivo* performance of the formulation.

Acknowledgements

This work is a part of Arik Dahan PhD dissertation. This study was supported by the Israeli Consortium of Pharmalogica. The authors thank Inbal Yifrach for

excellent technical assistance, and Dr. Josh Backon for constructive comments. A. Hoffman is affiliated with David R. Bloom Center of Pharmacy.

References

[1] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 46 (1–3) (2001) 3–26.
[2] M. Martinez, G. Amidon, A mechanistic approach to understanding the factors affecting drug absorption: a review of fundamentals, J. Clin. Pharmacol. 42 (6) (2002) 620–643.
[3] C.W. Pouton, Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and self-microemulsifying drug delivery systems, Eur. J. Pharm. Sci. 11 (Suppl. 2) (2000) S93–S98.
[4] A.J. Humberstone, W.N. Charman, Lipid-based vehicles for the oral delivery of poorly water soluble drugs, Adv. Drug Deliv. Rev. 25 (1) (1997) 103–128.
[5] P.P. Constantinides, Lipid microemulsions for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects, Pharm. Res. 12 (11) (1995) 1561–1572.
[6] A. Dahan, A. Hoffman, in: E. Touitou, B.W. Barry (Eds.), Enhancement in Drug Delivery, CRC Press, 2006, pp. 111–127.
[7] T. Gershanik, S. Benita, Self-dispersing lipid formulations for improving oral absorption of lipophilic drugs, Eur. J. Pharm. Biopharm. 50 (1) (2000) 179–188.
[8] H. Ljusberg-Wahren, F. Seier Nielsen, M. Brogard, E. Troedsson, A. Mullertz, Enzymatic characterization of lipid-based drug delivery systems, Int. J. Pharm. 298 (2) (2005) 328–332.
[9] C.J.H. Porter, W.N. Charman, In vitro assessment of oral lipid based formulations, Adv. Drug Deliv. Rev. 50 (Suppl. 1) (2001) S127–S147.
[10] N.H. Zangenberg, A. Mullertz, H. Gjelstrup Kristensen, L. Hovgaard, A dynamic in vitro lipolysis model: II: evaluation of the model, Eur. J. Pharm. Sci. 14 (3) (2001) 237–244.
[11] G.A. Kossena, B.J. Boyd, C.J.H. Porter, W.N. Charman, Separation and characterization of the colloidal phases produced on digestion of common formulation lipids and assessment of their impact on the apparent solubility of selected poorly water-soluble drugs, J. Pharm. Sci. 92 (3) (2003) 634–648.
[12] M. Hultin, A. Mullertz, M. Zundel, G. Olivecrona, T. Hansen, R. Deckelbaum, Y. Carpentier, T. Olivecrona, Metabolism of emulsions containing medium- and long-chain triglycerides or interesterified triglycerides, J. Lipid Res. 35 (10) (1994) 1850–1860.
[13] V.H. Sunesen, B.L. Pedersen, H.G. Kristensen, A. Mullertz, In vivo in vitro correlations for a poorly soluble drug, danazol, using the flow-through dissolution method with biorelevant dissolution media, Eur. J. Pharm. Sci. 24 (4) (2005) 305–313.
[14] J.B. Dressman, C. Reppas, In vitro–in vivo correlations for lipophilic, poorly water-soluble drugs, Eur. J. Pharm. Sci. 11 (Suppl. 2) (2000) S73–S80.
[15] A. Dahan, A. Hoffman, Use of a dynamic in vitro lipolysis model to rationalize oral formulation development for poor water soluble drugs: correlation with in vivo data and the relationship to intra-enterocyte processes in rats, Pharm. Res. 23 (9) (2006) 2165–2174.
[16] C.J.H. Porter, A. Taillardat-Bertschinger, B.J. Boyd, J.M. O'Connor, G.A. Edwards, W.N. Charman, Use of in vitro lipid digestion data to explain the in vivo performance of triglyceride-based oral lipid formulations of poorly water-soluble drugs: studies with halofantrine, J. Pharm. Sci. 93 (5) (2004) 1110–1121.
[17] N.A. Kasim, M. Whitehouse, C. Ramachandran, M. Bermejo, H. Lennernas, A.S. Hussain, H.E. Junginger, S.A. Stavchansky, K.K. Midha, V.P. Shah, G.L. Amidon, Molecular properties of WHO essential drugs and provisional biopharmaceutical classification, Mol. Pharm. 1 (1) (2004) 85–96.
[18] S.D. Mithani, V. Bakatselou, C.N. TenHoor, J.B. Dressman, Estimation of the increase in solubility of drugs as a function of bile salt concentration, Pharm. Res. 13 (1) (1996) 163–167.
[19] F.A. Alvarez Nunez, S.H. Yalkowsky, Correlation between logP and ClogP for some steroids, J. Pharm. Sci. 86 (10) (1997) 1187–1189.
[20] C.A.S. Bergstrom, U. Norinder, K. Luthman, P. Artursson, Experimental and computational, Pharm. Res. 19 (2) (2002) 182–188.
[21] A. Leo, C. Hansch, D. Elkins, Partition co-efficients and their uses, Chem. Rev. 71 (1971) 525–616.
[22] G.L. Amidon, H. Lennernas, V.P. Shah, J.R. Crison, A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability, Pharm. Res. 12 (3) (1995) 413–420.
[23] L. Sek, C.J.H. Porter, W.N. Charman, Characterisation and quantification of medium chain and long chain triglycerides and their in vitro digestion products, by HPTLC coupled with in situ densitometric analysis, J. Pharm. Biomed. Anal. 25 (3-4) (2001) 651–661.
[24] Hans Lenneras, Human intestinal permeability, J. Pharm. Sci. 87 (4) (1998) 403–410.
[25] S. Hess, V. Rotshild, A. Hoffman, Investigation of the enhancing mechanism of sodium *N*-[8-(2-hydroxybenzoyl)amino]caprylate effect on the intestinal permeability of polar molecules utilizing a voltage clamp method, Eur. J. Pharm. Sci. 25 (2–3) (2005) 307–312.
[26] B.I. Polentarutti, A.L. Peterson, A.K. Sjoberg, E.K.I. Anderberg, L.M. Utter, A.L.B. Ungell, Evaluation of viability of excised rat intestinal segments in the Ussing chamber: investigation of morphology, electrical parameters, and permeability characteristics, Pharm. Res. 16 (3) (1999) 446–454.
[27] R.M. Menon, W.H. Barr, Comparison of ceftibuten transport across Caco-2 cells and rat jejunum mounted on modified Ussing chambers, Biopharm. Drug Dispos. 24 (7) (2003) 299–308.
[28] A. Hoffman, G. Levy, Kinetics of drug action in disease states. XXIX. Effect of experimental nephrotic syndrome on the pharmacodynamics of heptabarbital: implications of severe hypoalbuminemia, J. Pharmacol. Exp. Ther. 249 (1) (1989) 117–122.
[29] J.O. Christensen, K. Schultz, B. Mollgaard, H.G. Kristensen, A. Mullertz, Solubilisation of poorly water-soluble drugs during in vitro lipolysis of medium- and long-chain triacylglycerols, Eur. J. Pharm. Sci. 23 (3) (2004) 287–296.
[30] A. Dokoumetzidis, P. Macheras, A century of dissolution research : from Noyes and Whitney to the biopharmaceutics classification system, Int. J. Pharm. 321 (1-2) (2006) 1–11.
[31] L. Sek, C.J. Porter, A.M. Kaukonen, W.N. Charman, Evaluation of the in-vitro digestion profiles of long and medium chain glycerides and the phase behaviour of their lipolytic products, J. Pharm. Pharmacol. 54 (1) (2002) 29–41.
[32] T.R. Bates, J.A. Sequeria, Bioavailability of micronized griseofulvin from corn oil-in-water emulsion, aqueous suspension, and commercial tablet dosage forms in humans, J. Pharm. Sci. 64 (5) (1975) 793–797.
[33] P.J. Carrigan, T.R. Bates, Biopharmaceutics of drugs administered in lipid-containing dosage forms. I. GI absorption of griseofulvin from an oil-in-water emulsion in the rat, J. Pharm. Sci. 62 (9) (1973) 1476–1479.
[34] A. Kaukonen, B.J. Boyd, W.N. Charman, C.J.H. Porter, Drug solubilization behavior during in vitro digestion of suspension formulations of poorly water-soluble drugs in triglyceride lipids, Pharm. Res. 21 (2) (2004) 254–260.
[35] M. Tomita, M. Hayashi, T. Horie, T. Ishizawa, S. Awazu, Enhancement of colonic drug absorption by the transcellular permeation route, Pharm. Res. 5 (12) (1988) 786–789.
[36] M. Lindenberg, S. Kopp, J.B. Dressman, Classification of orally administered drugs on the World Health Organization Model list of Essential Medicines according to the biopharmaceutics classification system, Eur. J. Pharm. Biopharm. 58 (2) (2004) 265–278.
[37] A. Dahan, A. Hoffman, Evaluation of a chylomicron flow blocking approach to investigate the intestinal lymphatic transport of lipophilic drugs, Eur. J. Pharm. Sci. 24 (4) (2005) 381–388.