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Quantitative Prediction of Oral Bioavailability of a Lipophilic Antineoplastic Drug Bexarotene Administered in Lipidic Formulation Using a Combined *In Vitro* Lipolysis/Microsomal Metabolism Approach

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ABSTRACT

For performance assessment of the lipid-based drug delivery systems (LBDDSs), *in vitro* lipolysis is commonly applied because traditional dissolution tests do not reflect the complicated *in vivo* micellar formation and solubilization processes. Much of previous research on *in vitro* lipolysis has mostly focused on rank-ordering formulations for their predicted performances. In this study, we have incorporated *in vitro* lipolysis with microsomal stability to quantitatively predict the oral bioavailability of a lipophilic antineoplastic drug bexarotene (BEX) administered in LBDDS. Two types of LBDDS were applied: lipid solution and lipid suspension. The predicted oral bioavailability values of BEX from linking *in vitro* lipolysis with microsomal stability for lipid solution and lipid suspension were 34.2 ± 1.6% and 36.2 ± 2.6%, respectively, whereas the *in vivo* oral bioavailability of BEX was tested as 31.5 ± 13.4% and 31.4 ± 5.2%, respectively. The predicted oral bioavailability corresponded well with the oral bioavailability for both formulations, demonstrating that the combination of *in vitro* lipolysis and microsomal stability can quantitatively predict oral bioavailability of BEX. *In vivo* intestinal lymphatic uptake was also assessed for the formulations and resulted in <1% of the dose, which confirmed that liver microsomal stability was necessary for correct prediction of the bioavailability.

Introduction

Modern drug discovery programs have resulted in the development of increased number of drug candidates with low aqueous solubility.[1] It is a general concept that the drug must be solubilized in the gastrointestinal (GI) tract to be able to access the enterocytes for permeation.[2] Poor aqueous solubility limits the rate of dissolution and consequently the amount of the drug that can be absorbed following oral administration. To overcome such situations, a range of formulation approaches has been studied including the use of lipids, surfactants, solid dispersions, and fabrication of nanoparticles.[3] Among them, the application of lipid-based drug delivery systems (LBDDSs), including self-emulsifying drug delivery system, has been successful in increasing the solubility and oral bioavailability (F_{oral}) as well as reducing the variability of oral absorption.[2,4,5]

The aim of most LBDDSs is to solubilize poorly soluble drugs in the formulation and then maintain the drug in a solution as it is administered into the GI tract.[2] As a result of this solubilization, the

Abbreviations used: BCS, biopharmaceutics classification system; BEX, bexarotene; ESI, electrospray ionization; GI, gastrointestinal; HPLC, high-performance liquid chromatography; LBDDS, lipid-based drug delivery systems; MRM, multiple reaction monitoring; PEG400, polyethylene glycol 400; SD, standard deviation; SEDDS, self-emulsifying drug delivery system.

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dissolution step of the drug in the GI tract can be avoided and therefore could promote absorption. For these reasons, it is a common practice to assess solubility of the drug in various lipids and surfactants during development of LBDDS.[3,6] It has to be noted however that solubilization of a drug during formulation processes does not always lead to solubilized drug under physiological conditions in the GI tract.[7] This is closely related to the complicated processes of LBDDS digestion and mixed micelles formation in the GI tract.[1,2] Due to this complexity, *in vitro* lipolysis or digestion systems are recommended to assess and predict the performance of LBDDS at physiological conditions.[8-10]

Traditionally, studies of performance assessment of LBDDS by *in vitro* lipolysis mostly provided rank order of the formulations for further development or achieving certain level of *in vitro–in vivo* correlations.[8,11-15] Recently, a novel approach of a combined *in vitro* lipolysis with microsomal metabolism was developed in our laboratory by Benito-Gallo et al.,[8] which provided an opportunity for quantitative prediction of F_{oral} of drugs administered in LBDDSs. However, the concept of *in vitro* lipolysis/microsomal metabolism link was developed using only 2 model compounds and warrants validation with additional compounds. In addition, the pharmacokinetic data used to develop the combined *in vitro* lipolysis/microsomal metabolism approach were obtained from literature, which was an additional limitation of the previous work.[8] Therefore, in the present study, we show that *in vitro* lipolysis linked with microsomal stability can quantitatively predict the F_{oral} of bexarotene (BEX, structure shown in Fig. 1), an antineoplastic compound, when administered orally in LBDDS in rats. In addition, the validation of the predictions was achieved in this work by conducting *in vivo* bioavailability and intestinal lymphatic transport studies. The information on lymphatic transport is important as drugs that have substantial intestinal lymphatic transport avoid liver at the first pass, and therefore, hepatic microsomal metabolism element of quantitative prediction could be omitted.

Materials and Methods

Materials

BEX was obtained from LC Laboratories (Woburn, MA). Linoleic acid was purchased from Acros Organics (Loughborough, UK). Trizma maleate, $MgCl_2$, KH_2PO_4, K_2HPO_4, and reduced nicotinamide adenine dinucleotide phosphate (NADPH), porcine pancreatin powder (8 × United States Pharmacopeia specifications), sodium taurocholate, NaCl, lecithin, tetrabromo-o-cresol, and sunflower oil were from Sigma (Gillingham, UK). Calcium chloride was from Alfa Aesar (Lancashire, UK). Pooled male rat liver microsome was purchased from Gibco (Paisley, UK). Polyethylene glycol 400 (PEG400) was obtained from Fisher Scientific (Loughborough, UK). All solvents used were of high-performance liquid chromatography (HPLC) grade or higher.

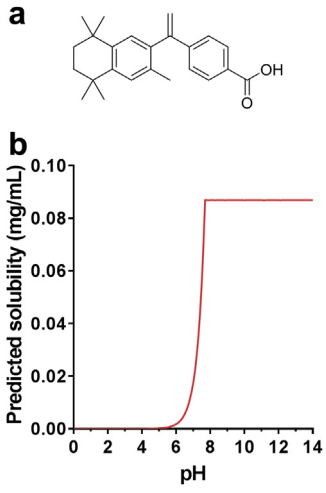

Figure 1. Chemical structure of bexarotene (a) and pH-dependent solubility predicted by GastroPlus™ (b).

Solubility

Aqueous solubility of BEX at different pH was predicted by GastroPlus™ version 9.6.00015 with built-in ADMET Predictor™ v9.0.0.0. Reference solubility at pH 7.0 in water of 50 μM was given as input.[16] Solubility of BEX in various vehicles was measured following a previously reported method with minor modifications.[17] In glass vials, BEX (10 mg) was mixed with 1 mL of PEG400, linoleic acid, or sunflower oil. The mixture was stirred magnetically for 72 h at 37°C and then was filtered using a Costar Spin-X Centrifuge Tube (0.22 μm pore size; Fisher Scientific) at 2400 × *g* for 5 min. The filtrate was collected and subjected for analysis using HPLC-ultraviolet (UV). The experiment was conducted in triplicate.

In Vitro *Lipolysis*

In vitro lipolysis was performed based on the method that was previously validated and reported.[8,18,19] The lipolysis digestion buffer was composed of the following: 50 mM Tris maleate; 150 mM NaCl; 5 mM $CaCl_2$, 5 mM sodium taurocholate; and 1.25 mM lecithin. The pH was adjusted to 6.8 before the experiment. BEX was formulated using linoleic acid or sunflower oil at 4 mg/mL and was added to the digestion buffer. The lipolysis was initiated by addition of the enzyme solution prepared from pancreatin extract and the pH of the reaction mixture was maintained at 6.8 using a pH-stat titrator (T50 Graphix with DG111-SC pH probe, Mettler Toledo Inc.) and stirred at 37°C. Following completion of lipolysis, the mixture was subjected to ultracentrifugation at 268,350 × *g* for 90 min at 37°C (SORVALL® TH-641 Rotor, Thermo Fisher Scientific, UK). The lipid, micellar, and sediment phases were collected and prepared for analysis using HPLC-UV. After analysis of samples from each phase, the fraction of drug found in each phase was determined. The concentration of BEX in the micellar phase was used to calculate the fraction predicted to be absorbed ($F_{abs,\ predicted}$) using equations reported previously[8]:

$$F_{abs,predicted} = [Drug]_{MP} \cdot \frac{40\ mL}{0.3\ mL \cdot 4\ mg/mL}$$

where, $[Drug]_{MP}$ is the drug concentration (mg/mL) found in the micellar phase, 40 mL is the volume of the *in vitro* lipolysis buffer, and 0.3 mL of the 4 mg/mL BEX formulation was used. Experiment was performed in triplicate.

Liver Microsomal Stability

Liver microsomal metabolic stability assay was performed using rat liver microsome following previously reported methods with minor modifications.[8,20] The reaction mixture was composed of the followings: 0.5 mg microsomal protein per mL; 10 mM $MgCl_2$; 1 mM NADPH; and 84.7 mM potassium phosphate buffer at pH 7.4. BEX was tested at 1 μM, and the reaction was initiated with the addition of NADPH. Samples were withdrawn at predetermined time points, and reaction was terminated by excessive volume of acetonitrile. Samples were analyzed by HPLC-UV and performed in triplicate.

Half-life ($t_{1/2}$) of BEX was obtained from the semi-log plot of concentration-time profile:

$$t_{1/2} = -\frac{0.693}{k}$$

where, k is the slope obtained by plotting natural log percentage of BEX versus time. The intrinsic clearance was then obtained by the following equation[21,22]:

$$CL_{int} = \frac{0.693}{t_{1/2}} \cdot \frac{mL\ incubation}{mg\ microsomes} \cdot \frac{mg\ microsomes}{g\ liver} \cdot \frac{g\ liver}{kg\ body\ weight}$$

where, mg microsomes/g liver and g liver/kg body weight values were 44.8 and 40.0, respectively, for rats.[23] The hepatic clearance was then obtained by using parallel-tube model[21,22]:

$$CL_h = Q \cdot \left(1 - e^{(-CL_{int}/Q)}\right)$$

where, Q is the hepatic blood flow rate of 55.2 mL/min/kg for rats.[23] The fraction that escapes hepatic metabolism (F_h) was then calculated using the following equation[21,22]:

$$F_h = 1 - \frac{CL_h}{Q}$$

The F_h obtained from the above equation also represents the fraction that escapes hepatic first-pass effect during oral absorption.

Calculation of Predicted Oral Bioavailability

By incorporating *in vitro* lipolysis and *in vitro* metabolic stability results, predicted oral bioavailability ($F_{oral,\ predicted}$) was calculated using the following equation[8]:

$$F_{oral,predicted} = F_{abs,predicted} \cdot F_h$$

Animal Experiments

Animals

Procedures and protocols of all animal experiments in this study were approved by the Animal Care Committee of Sungkyunkwan University (School of Pharmacy) and performed in accordance with National Institutes of Health guidelines (NIH publication No. 86-23, revised 1985). Male Sprague-Dawley rats (aged 8 weeks, body weight 238-274 g) were purchased from Samtako Co., (Osan, Gyeonggi-do, South Korea). Rats were kept in clean plastic cages with freely accessible standard rat diet (Samtako Co.) and water. The animals were housed at a temperature of 22 ± 2°C with a 12 h light-dark cycle and a relative humidity of 55 ± 10% and were acclimatized for at least 1 week before any procedures.

In Vivo *Plasma Pharmacokinetics*

The pharmacokinetics of BEX was characterized in rats after intravenous and oral administrations. Before surgery, the animals were anesthetized by intraperitoneal injection of Zoletil® 50 (Virbac Laboratories, Carros, France) (22.5 mg/kg) and cannulated with a polyethylene tubing (0.58 mm i.d., 0.96 mm o.d., Natsume, Tokyo, Japan) in the femoral and jugular veins for the intravenous administration group or in the jugular vein only for the oral administration group. Following the surgery, animals were kept in warm, clean cages for recovery for 24 h. For intravenous administration, BEX dissolved in PEG400 was injected into the femoral vein cannula at a dose of 5 mg/kg with an injection volume of 1 mL/kg. For oral administration, BEX formulated in linoleic acid or sunflower oil (4 mg in 1 mL for both formulations) was administered by oral gavage at a dose of 10 mg/kg with dosing volume of 2.5 mL/kg. Blood samples (0.1 mL) were collected from the jugular vein cannula at predetermined time points, and plasma samples were harvested by centrifugation at 16,000 × *g* for 5 min at 4°C and stored at −20°C until analysis.

In Vivo *Lymphatic Uptake*

Lymphatic delivery of BEX was characterized in rats after oral administration. Before surgery, the animals were given corn oil (1 mL) by oral gavage to facilitate mesenteric lymph duct cannulation. Approximately 2 h later, the rats were anaesthetized by intraperitoneal injection of Zoletil® 50 (Virbac Laboratories, Carros, France) (22.5 mg/kg), and the right side of the flank was shaved by an electric clipper and sterilized by 70% ethanol solution. The mesenteric lymph duct was exposed by incision of the right abdomen. After punctuation of the duct, a polyethylene tubing (0.58 mm i.d., 0.96 mm o.d., Natsume) was cannulated. The cannula was fixed and adhered with the use of cyanoacrylate glue. After cannulation, the wound was closed by suture and surgical clips. The animals were then kept in warm, clean cages for recovery for 2 h. For oral administration, BEX dissolved in PEG400, linoleic acid or sunflower oil was administered to 3 groups of rats by oral gavage at a dose of 10 mg/kg with 2.5 mL/kg dosing volume. The lymph fluid was continuously collected from the cannula, and the collection tube was changed at predetermined intervals. Collected lymph samples were stored at −20°C until analysis.

Analytical Methods for Determination of Concentration Levels

Determination of BEX in Samples From In Vitro *Experiments*

Samples from *in vitro* experiments were analyzed based on a previously reported HPLC-UV method[24] with minor modifications. Modifications included using a flow rate of 0.4 mL/min and the use of hexane (3 mL) as the extraction solvent. The range of calibration curves was also adjusted to 500-20,000 ng/mL.

Determination of BEX in Samples From In Vivo *Experiments*

An API 2000 mass spectrometer coupled with a Waters 2690 separation module was used for sample analysis. Separation was achieved on a Kinetex biphenyl column (100 × 2.1 mm, 2.6 μm; Phenomenex, Torrance, CA). The column oven temperature was 40°C, and the flow rate was 0.25 mL/min. The total run time was 8 min, and the data were processed by analyst version 1.4.0 (AB Sciex, Framingham, MA).

The electrospray ionization source was operated in the negative mode. The multiple reaction monitoring parameters and MS/MS conditions were as follows: m/z 347.1 → 303.4 for BEX; m/z 422.78.9 for tetrabromo-o-cresol (internal standard); curtain gas: 25 psig; collision gas: 5 psig; ion spray voltage: −4500 V; ion source temperature: 400°C; ion source gas 1: 20 psig; ion source gas 2: 40 psig; declustering potential: −41 V; focusing potential −350 V; entrance potential: −12 V; collision energy: −30 eV; collision cell exit potential: −28 eV.

Both plasma and lymph samples were prepared by protein precipitation with acetonitrile. Samples (50 μL) were added with the internal standard solution (100 μL, 500 ng/mL tetrabromo-o-cresol in acetonitrile) and additional acetonitrile of 100 μL. The mixture was vortex-mixed for 10 s and then centrifuged for 5 min at 16,000 × *g*. The supernatant (70 μL) was then mixed with 130 μL of water and transferred to a HPLC vial. A portion (15 μL) of the

mixture was injected into the liquid chromatography-tandem mass spectrometry.

Statistical Analyses

All data were presented as mean ± standard deviation. Two-tailed unpaired t-test was applied to determine statistical significance and a p-value of <0.05 was considered significant. When more than 2 groups were compared, a one-way analysis of variance followed by Tukey's multiple comparisons test was used. GraphPad Prism version 7.01 (GraphPad Software, Inc., La Jolla, CA) was used for statistical analysis. Noncompartmental analysis using Phoenix WinNonlin 6.3 software (Pharsight, Mountain View, CA) was applied to calculate the pharmacokinetic parameters from plasma concentration-time profiles.

Results

The predicted pH-dependent aqueous solubility profile of BEX is shown in Figure 1. Although it was predicted to be slightly higher in basic pH conditions, the solubility in overall was predicted to be < 0.1 mg/mL. It was in agreement with the fact that BEX is a class II drug of the Biopharmaceutics Classification System and therefore BEX would benefit with application of LBDDSs.[16,25]

Solubility assessment results of BEX in linoleic acid and sunflower oil are shown in Figure 2. The solubility of BEX in linoleic acid was 6.2-fold higher than that in sunflower oil. It should be noted that the solubility in sunflower oil was <4 mg/mL, and hence, the formulation of BEX in sunflower oil used for *in vitro* lipolysis and *in vivo* pharmacokinetic experiments was a lipid suspension. Solubility of BEX in linoleic acid was >4 mg/mL, and therefore, the formulation tested in the experiment was a clear solution.

Both formulations of BEX in linoleic acid and sunflower oil were tested for their performance in *in vitro* lipolysis system (Fig. 3). Interestingly, both formulations resulted in comparable fractions of the drug found in the micellar phase, although formulation of linoleic acid was a clear solution and that of sunflower oil was a suspension. It showed that the concentration of BEX in the micellar phase is comparable regardless of their solubilized state in the formulation.

The results of liver microsomal stability of BEX performed using rat liver microsome is shown in Table 1. The parameters of intrinsic clearance, hepatic clearance, and F_h were calculated from the half-life obtained from the stability test, and the obtained F_h indicated that BEX would be classified as a moderately extracted compound.[26]

In vivo plasma pharmacokinetic profiles were determined in rats following intravenous and oral administrations. The profiles are shown in Figure 4, and pharmacokinetic parameters derived from the profiles are shown in Table 2. Although the maximum concentration observed for oral administration profile differed between the 2 formulations following oral administration, the overall exposure, determined by the area under the curve to the time extrapolated to infinity, did not differ significantly. Therefore, the F_{oral} was comparable between the 2 formulations. The elimination half-life was also not significantly different between the formulations.

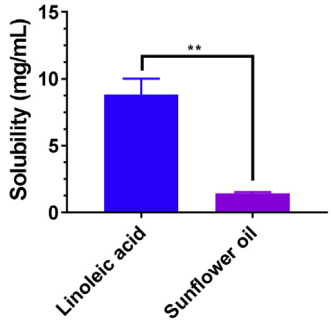

Figure 2. Solubility assessment of bexarotene in linoleic acid and sunflower oil (mean ± SD, $n = 3$). **, $p < 0.05$.

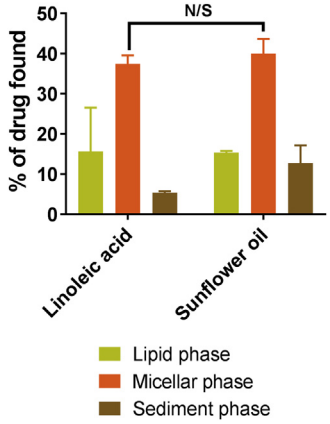

Figure 3. *In vitro* lipolysis assessment of bexarotene in formulations of linoleic acid (solution) and sunflower oil (suspension). The amount of drug was analyzed in lipid, micellar, and sediment phases (mean ± SD, $n = 3$). N/S, not significant.

Following the method of the previously reported study,[8] 2 predicted values were obtained: the $F_{abs,\ predicted}$ and the $F_{oral,\ predicted}$. The $F_{oral,\ predicted}$, which incorporates results of *in vitro* lipolysis and liver microsomal stability, resulted in comparable values to the *in vivo* experimental F_{oral} values (Table 2). It demonstrated that F_{oral} of BEX achievable by LBDDS can be quantitatively predicted by application of *in vitro* lipolysis linked with microsomal stability.

The intestinal lymphatic transport of BEX resulting from the formulations was tested with mesenteric lymph duct cannulated rats (Fig. 5). For this purpose, a lipid-free vehicle (PEG400) was also tested, and it was shown that both formulations did not improve lymphatic uptake of BEX compared with the lipid-free vehicle.

Discussion

In vitro lipolysis experiments are commonly used in assessment of LBDDS because the performance of LBDDS can be complicated by physiological processes of lipid digestion and therefore simple dissolution tests are often not applicable.[1,27] In general, in the *in vitro* lipolysis studies, the amount of the drug in the micellar fraction is considered to have the most relevance to oral absorption.[8] This is because the micellar phase consists of mixed micelles with the solubilized drug, which represents the fraction readily available for absorption. The lipid fraction contains the undigested lipids, and the sediment fraction is what has precipitated during the lipolysis; therefore, the drug in these 2 fractions is not readily available for absorption. Both formulations of linoleic acid and sunflower oil resulted in comparable fraction of BEX in the micellar phase following lipolysis (Fig. 3) and therefore were predicted to have comparable fraction absorbed ($F_{abs,\ predicted}$, Table 2). These

Table 1
Liver Microsomal Metabolic Stability Results of Bexarotene (Mean ± SD, $n = 3$)

Half-Life (min)	CL_{int} (mL/min/kg)	CL_h (mL/min/kg)	F_h (%)
62.8 ± 14.1	40.8 ± 8.9	28.6 ± 4.3	48.1 ± 7.8

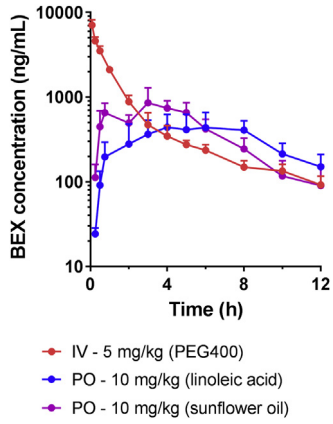

Figure 4. Plasma concentration-time profiles of BEX following intravenous administration at 5 mg/kg (in PEG400) and oral administration at 10 mg/kg (in linoleic acid or sunflower oil) in rats (mean ± SD, $n = 5$).

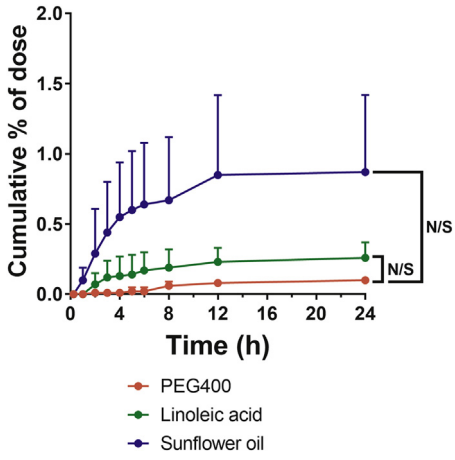

Figure 5. Cumulative intestinal lymphatic uptake of BEX from different formulations in 24 h following oral administration in the mesenteric lymph duct cannulated rats (mean ± SD, $n = 3$). N/S, not significant.

results indicate that the performance of the 2 formulations following oral administration would be at similar levels.

The $F_{abs,\ predicted}$ was then incorporated with F_h values from microsomal stability tests to predict oral bioavailability ($F_{oral,\ predicted}$), hence reflecting both absorption and hepatic first-pass effect. The $F_{oral,\ predicted}$ values for the 2 formulations shown in Table 2 corresponded to the F_{oral} values obtained from *in vivo* pharmacokinetic experiments, which demonstrates that the approach of linking *in vitro* lipolysis with microsomal stability can quantitatively predict the F_{oral} of BEX following its administration in LBDDS.

The quantitative prediction of F_{oral} was shown to be successful for 2 types of LBDDS in this study: lipid suspension (sunflower oil) and lipid solution (linoleic acid) (Table 2). Although sunflower oil was not able to fully solubilize BEX at 4 mg/mL, it interestingly resulted in comparable performance in *in vitro* lipolysis with linoleic acid formulation in which BEX was fully solubilized (Fig. 3). Moreover, it was remarkable that the 2 formulations resulted in comparable *in vivo* F_{oral} (Table 2), although a suspension would have had an additional dissolution step included in the solubilization processes of the drug. This highlights the fact that the *in vitro* lipolysis offers a more biorelevant performance assessment of LBDDS than traditional dissolution and that the *in vitro* lipolysis/microsomal metabolism link approach can be applied to different types of LBDDS.

Table 2
Pharmacokinetic Parameters of BEX Obtained From Plasma Concentration-Time Profiles Following Intravenous Administration at 5 mg/kg (in PEG400) and Oral Administration at 10 mg/kg (in Linoleic Acid or Sunflower Oil) in Rats (Mean ± SD, $n = 5$)

Parameters	PEG400 (IV)	Linoleic Acid (Oral)	Sunflower Oil (Oral)
T_{max} (h)	–	6.0 ± 3.1	3.0 ± 1.4
C_0 or C_{max} (ng/mL)	8834 ± 1842	533.6 ± 179.7	1002 ± 302.1
AUC_{all} (ng·h/mL)	8160 ± 212	5081 ± 2441	5210 ± 793
AUC_{inf} (ng·h/mL)	8812 ± 459	5553 ± 2354	5531 ± 917
$t_{1/2}$ (h)	4.5 ± 2.2	3.8 ± 2.3	3.0 ± 1.2
F_{oral} (%)	–	31.5 ± 13.4	31.4 ± 5.2
$F_{abs,\ predicted}$ (%)	–	71.1 ± 3.4	75.3 ± 5.4
$F_{oral,\ predicted}$ (%)	–	34.2 ± 1.6	36.2 ± 2.6

$F_{abs,\ predicted}$ and $F_{oral,\ predicted}$ were calculated using equations from reference with assumption that metabolism in the gut wall is negligible.[8]
T_{max}, time of maximum concentration observed; C_0, concentration extrapolated to time zero for intravenous administration profile; C_{max}, maximum concentration observed for oral administration profile; AUC_{all}, area under the curve to the last time point observed; AUC_{inf}, area under the curve to the time extrapolated to infinity; $t_{1/2}$, elimination half-life.

Although *in vitro* lipolysis has been mainly used for digestible lipids, as the experimental system contains lipase enzyme, we here show that it can also be applied to formulations of lipid digestion product (or indigestible lipid). Linoleic acid used in this study is a free fatty acid which is in fact one of the products of lipid digestion and the applicability of *in vitro* lipolysis is demonstrated by the $F_{oral,\ predicted}$ corresponding with the experimental *in vivo* F_{oral} (Table 2). The presence of lipids or digestive products, including free fatty acids, in the GI tract itself can induce release of the cholecystokinin, which stimulates secretion of pancreatic enzymes and bile acids.[2,28] The *in vitro* lipolysis system mimics these endogenous components, and therefore, it better mimics the environment of the GI tract. Although indigestible lipids and lipid digestion products would not benefit from the "lipolysis" process, the *in vitro* lipolysis system as a whole provides more biorelevance in assessment of their ability to facilitate mixed micelle formation and hence drug solubilization.

It should be noted that BEX has logP of 7.28 (ACD/Labs, Toronto, Canada) and belongs to class II of the Biopharmaceutics Classification System.[25] Accordingly, the F_{oral} of BEX would be more dependent on solubility in the GI tract rather than permeability across membranes. Therefore *in vitro* lipolysis results were sufficient to predict the absorbed fraction without consideration of permeability which was in accordance with previous studies for similar compounds.[8,12,13]

The results in Figure 5 show that the intestinal lymphatic uptake did not differ significantly between the 2 formulations, and in fact not different from a lipid-free vehicle. LBDDS are often used to enhance intestinal lymphatic delivery of lipophilic drugs for the purpose of increasing the F_{oral} and targeting the intestinal lymphatic system.[3,28-30] It has been previously suggested that it is the inherent physicochemical properties of the drug that determines the association ability of the drug with chylomicrons, which eventually governs intestinal lymphatic transport.[17,31] The intestinal lymphatic transport of BEX, with its low chylomicron association reported previously,[32] was not affected by LBDDS. It confirms the relevance of application of liver microsomal stability in bioavailability prediction as minimal lymphatic transport would mean that hepatic first-pass effect would be applied to BEX.[8] When hepatic first-pass effect is applied to the absorbed drug, *in vitro* lipolysis system alone would not be able to predict the F_{oral} accurately. Therefore, it becomes evident that liver microsomal stability studies needed to be linked with *in vitro* lipolysis to quantitatively predict the F_{oral} of BEX.

In conclusion, we have shown that F_{oral} of LBDDS can be quantitatively predicted by incorporation of *in vitro* lipolysis and microsomal stability. The evaluations and predictions were applied to formulations of a lipid suspension and a lipid solution, which resulted in comparable *in vitro* and *in vivo* performance. The predictability of the approach was found to be acceptable for the 2 different types of LBDDSs. To make a head-to-head comparison, experimental bioavailability obtained from our own *in vivo* pharmacokinetic studies was used. In addition, intestinal lymphatic transport was assessed for the formulations to confirm that microsomal stability results need to be linked with *in vitro* lipolysis for the F_{oral} prediction.

Acknowledgments

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