Regional-Dependent Intestinal Absorption and Meal Composition Effects on Systemic Availability of LY303366, a Lipopeptide Antifungal Agent, in Dogs

C. LI,[1] D. FLEISHER,[1] L. LI,[1] J. R. SCHWIER,[2] S. A. SWEETANA,[2] V. VASUDEVAN,[2], L .L. ZORNES,[2] L-H. PAO,[1†] S. Y. ZHOU,[1‡] R. E. STRATFORD[2]

[1] 3058 College of Pharmacy, University of Michigan, Ann Arbor, Michigan 48109-1065

[2] Eli Lilly and Company, Indianapolis, Indiana 46285

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ABSTRACT: Low oral bioavailability and a negative meal effect on drug plasma levels motivated studies on formulation and meal composition effects on the absorption of LY303366, a poorly water-soluble, semisynthetic, cyclic peptide antifungal drug. Solid drug particle size and meal composition studies were evaluated in beagle dogs. Canine regional absorption studies were also carried out utilizing surgically implanted intestinal access ports, and Caco-2 studies were performed to evaluate drug candidate intestinal permeability.

Particle size and Caco-2 data indicate that drug permeability limitations to absorption are more important than dissolution rate limits. Caco-2 cell-associated LY303366 approached 10% of incubation concentration that is in the range of the oral bioavailability of the drug. Canine regional absorption studies showed that the extent of LY303366 absorption following duodenal administration was similar to that following oral administration. Significantly lower drug plasma levels were obtained following administration through a colonic access port, a result consistent with poor membrane permeation. Administration of drug with meals of mixed composition, as well as simple fat and protein meals, resulted in significant reductions in AUC_{0-48h} compared with results from fasted dogs. In contrast, carbohydrate meals did not reduce drug plasma levels compared to controls. Intravenous pretreatment with devazepide, a cholecystokinin (CCK) antagonist that blocks canine biliary secretion, did not reverse the negative effect of the fat meal on LY303366. Taken together, the results from the present study suggest that membrane-permeability-limited absorption is the cause of the observed regionally dependent absorption of LY303366 in the dog and that the observed negative meal effects depend on composition but are independent of biliary secretion.
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Keywords: intestinal absorption; intestinal permeability; antifungal drug; meal and food effects

INTRODUCTION

LY303366, a cyclic lipopeptide, is a semisynthetic analogue of Echinocandin B (ECB) that is being developed as an antifungal agent. LY303366 shows *in vitro* and *in vivo* activity against Candida, Aspergillus,[1] and Pneumocystis[2] microorganisms. The antifungal potency of LY303366 is excellent, with a minimum inhibitory concentra-

Present address: Schering Plough Research, Kenilworth, NJ 07033-1300

†*Present address:* School of Pharmacy, National Defense Medical Center, Taipei, Taiwan

‡*Present address:* Drug Metabolism, Pfizer Central Research, Groton, CT 06340

Correspondence to: R. E. Stratford (Telephone: 317-276-4190; Fax: 317-276-7040; E-mail: stratford_robert_e_jr@lilly.com)

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tion (MIC) versus *C. albicans* of 0.005 μg/mL *in vitro*. In a mouse model of *C. albicans* infection, the ED_{50} of LY303366, which is the dose at which 50% of animals survive 7 days, was 0.3 and 7.8 mg/kg for intravenous (iv) and oral administration routes, respectively. The echinocandin lipopeptides most likely owe their primary mode of antifungal activity to their inhibition of the biosynthesis of the fungal cell wall.[3] LY303366 has a molecular weight of 1140.3 and is poorly soluble in water (≤0.1 mg/mL), as well as over the pH range 4–9 (≤0.1 mg/mL). It has a log octanol–water partition coefficient of 2.0. The chemical structure of LY303366 is shown in Figure 1.

As part of an oral single dose clinical trial evaluation of LY303366 safety, a negative food effect (reduced drug plasma levels as compared with administration with water) was observed. Specifically, concurrent administration of a 50-mg dose with a high-fat meal resulted in a 75% reduction in $AUC_{0-\infty}$ (area under the curve of concentration versus time from time zero to infinity). Coadministration of a 700-mg dose with a low-fat meal resulted in a 35% reduction in $AUC_{0-\infty}$.[4] In a preliminary evaluation conducted in dogs, a 30% decline in both $AUC_{0-\infty}$ and C_{max} (maximum concentration) was observed when a 250-mg dose of LY303366 was coadministrated with a solid meal.[5] At this same dosage, the oral bioavailability in dogs administered LY303366 in the fasted state was ~5%[6] to 10%.[7] In this report, formulation studies and Caco-2 permeation studies were carried out to assess potential rate limits to drug absorption. Meal composition studies were conducted in dogs to further explore the negative meal effect. The fact that carbohydrate meals did not cause a negative effect whereas lipid and protein meals did result in decreases in LY303366 absorption prompted a limited set of mechanistic studies to evaluate the impact of inhibition of biliary secretion. When lipid and protein are administered, the digestion products within the upper small intestine induce secretion of biliary and pancreatic fluids that dramatically alter the luminal environment. Bile salts have been reported to increase the absorption of some hydrophilic compounds by enhancing the permeability of the intestinal membrane and some lipophilic compounds by micellar solubilization.[8,9] However, bile salts have also been reported to reduce the amount of solute in solution available for absorption of other drugs, which could underlie a negative food effect with lipid and protein meals.[10,11]

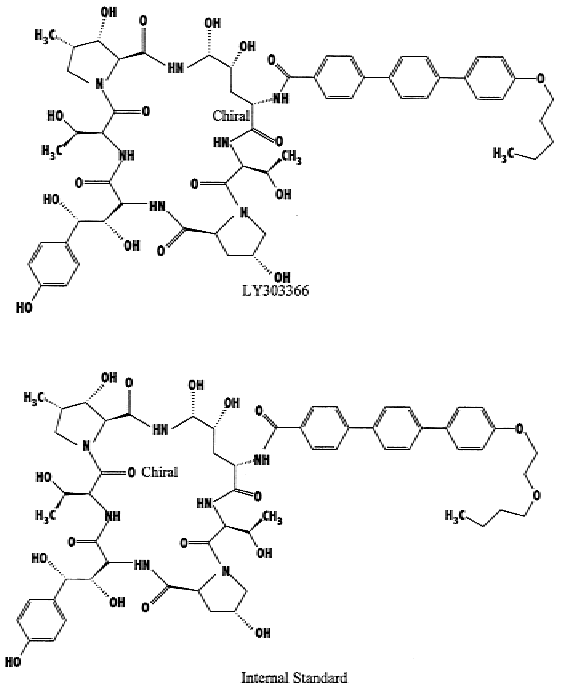

Figure 1. Chemical structure of LY303366 and its internal standard.

EXPERIMENTAL SECTION

Materials

^{14}C-LY303366 and ^{3}H-mannitol were from Amersham Life Sciences, Arlington Heights, IL. Eli Lilly and Company (Indianapolis, IN) supplied LY303366 parenteral solution (10 mg/ mL). LY303366 standard compound and its internal standard (306168, Figure 1) were also from Eli Lilly and Company. All other materials were commercially available unless stated.

Caco-2 cells were obtained by permission from the Memorial Sloan-Kettering Cancer Center and were cultured at 37 °C in a humidified atmosphere of 5% CO_2 in air. They were grown in DMEM:F12 (3:1) media supplemented with 20 mM HEPES, 5% fetal bovine serum, and 50 μg/mL tobramycin. Monolayers at 75–90% confluency were either subcultured on a weekly basis, using a 1:10 split ratio, or seeded onto Millicell-PCF® polycarbonate inserts (30-mm diameter, 0.4-μm pore size; Millipore Corp., Bedford, MA), at a density of 600,000 cells per filter, and fed every other day. For transport studies, cells from passages 40–42 were used following 21–30 days post-seeding. Transepithelial electrical resistance

(TEER) was measured immediately prior to an experiment using the Millicell-ERS system (Millipore Corp.). Cells with a net resistance < 300 $\Omega \cdot cm^2$ were not used.

The reference dexamethasone permeability in this system was determined under the following experimental conditions. Initial donor dexamethasone concentration was 25 μM traced with 0.5 μCi/mL ^{3}H-dexamethasone. Cold stock solutions were made up at 2.5 mM with dimethylsulfoxide (DMSO) so that the final DMSO concentration in the donor compartment was 1%. Initial dexamethasone flux was determined over 180 min, providing sink conditions, and the receiver compartment was sampled at 30-min intervals.

Animal Studies

Studies in dogs adhered to the "Principles of Laboratory Animal Care" in accordance with NIH publication #85-23 and were approved by the University Committee on Use and Care of Animals.

Formulation Effects in Fasted Dogs

Using a non-crossover design, 4 fasted beagle dogs were administered a 250-mg (2 × 125-mg capsules) dose containing LY303366 with a mean particle size of either 11 or 154 μm. Then, 50 mL of water was coadministered with the capsules. The 11-μm particle sized material was produced from the larger particle size using a standard alpine mill. Blood samples were taken up to 48 h after administration of drug.

Particle Size Determination

Equivalent spherical volume diameters were measured using a Horiba LA910 Laser Scattering Particle Size Distribution Analyzer. Solid drug sample was suspended in 180 mL of dispersant solution. Solid sample was added to the dispersant until an acceptable level of laser light obscuration was achieved, at which point the particle size distribution was measured. Prior to the sample suspension, the dispersant solution was prepared using 0.1% (w/v) SPAN 80 in cyclohexane. After presaturation with the drug substance, the dispersant solution was filtered through a 0.2-μm microporous membrane filter to provide the necessary particle-free suspending dispersant. The 154-μm (mean) drug sample showed 90% particles cumulatively <315 μms (d90) and 10% cumulatively <17 μms (d10). The 11-μm (mean) drug sample showed 90% <19 μms (d90) and 10% <4 μms (d10). Although a standard deviation was not obtained, the graphical distributions clearly represented two different particle size populations. Additionally, the 154-μm (mean) sample showed a BET (Brunauer–Emmett–Teller) surface area of 0.44 m^2/g, whereas the 11-μm (mean) sample showed a BET surface area of 2.0 m^2/g.

In Vitro Dissolution Studies

Capsule dissolution was reported using the average of six capsules run via a modified USP paddle method (paddle speed of 100 rpm) in 900 mL of 1.0% (w/v) polysorbate 80 in purified water maintained at 37 °C. Each capsule contained 125 mg of LY303366. Analysis was conducted using reversed-phase high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection (220 nm). The surfactant was necessary to solubilize sufficient amounts of the active pharmaceutical ingredient.

Caco-2 Permeability Studies

Measurements of transepithelial fluxes of ^{14}C-LY303366 and ^{3}H-mannitol were made simultaneously at 37 ° C using a side-by-side diffusion chamber described previously.[12] The transport medium consisted of 25 mM HEPES buffer, pH 7.4, along with 125 mM NaCl, 5.2 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM glucose, and 0.1% bovine serum albumin. To initiate an experiment, 200 μL of a stock solution of LY303366 and mannitol in 10% water/90% ethanol was added to either the apical (14 mL final volume) or basal (13 mL final volume) side of the monolayer. The final concentration of ethanol was 1.3% (v/v). Samples (200 μL) were taken from the contralateral compartment at various times up to 120 min and replaced with an equal volume of fresh transport buffer. At the beginning and end of an experiment, samples were taken from the donor chamber to determine permeability and mass balance, respectively. Rates of transport under initial rate conditions (<10% transport) were determined by linear regression analysis; these rates were divided by the initial donor concentration to calculate the corresponding permeability coefficients. Preliminary studies designed to assess the stability of LY303366 when subjected to the incubation conditions just described indicated an LY303366 recovery of ≥95% between the concentrations of 1

to 6 μM. The concentration range evaluated was limited by the solubility of LY303366.

At the conclusion of an incubation, filters were removed, washed twice with 2 mL of cold pH 7.4 transport buffer, and subsequently transferred to10 mL of liquid scintillation cocktail for the determination of bound radioactivity. Mass balance was calculated by dividing the sum of (1) cell associated radioactivity, (2) the cumulative amount of radioactivity transported over the duration of the incubation, and (3) the final donor radioactivity by the initial donor chamber radioactivity. For LY303366, mass balance was on average 91 ± 5.3%; average mannitol mass balance was 93 ± 5.4% ($n = 29$).

Regional-Dependent Studies

Bolus oral and colonic administration of 25 mL of LY303366 solution (250-mg dose) was carried out in four male Beagle Dogs, weighing 9.5–13.6 kg, utilizing a non-crossover design. Duodenal and mid-jejunal bolus administration of the same dose and volume were also conducted in three dogs and one dog, respectively. Upper and lower intestinal access ports (Access Technologies, Skokie, IL) were surgically implanted. The washout period between each study was at least 1 week. In the oral study, LY303366 solution was administrated directly into the stomach through a gastric tube. In the regional study, drug solution was pushed as a bolus administration through an intestinal access port either directly into the small intestine or colon. Blood samples were taken up to 48 h after administration of drug.

Meal Effect Studies

Meal effect and subsequent mechanistic studies were carried out utilizing a non-crossover design. Fasted and hyper-osmolal solution treatment controls consisted of 250 mg of drug coadministered with 240 mL of either water or sodium chloride solution (290 ± 20 mOsm/kg), respectively. Test meals included a liquid mixed-composition meal (Ensure®, 250 kcal/240 mL), and fat (20% Intralipid®), protein (CASEC® Calcium Caseinate), sucrose, and maltodextrin (Moducal®) meals, which are all commercially available. Each meal was prepared to have a final total volume of 240 mL and meal osmolality was adjusted to 480 ± 20 mOsm/kg with NaCl when needed. The total calories were 250 kcal for Ensure® and Intralipid® meals, and 125 kcal for protein, sucrose, and maltodextrin meals. The selection of a lower calorie protein meal compared with the higher calorie mixed and lipid meals was motivated by a desire to study low viscosity liquid meals. Following an overnight fast, LY303366 mixed with control solutions or meal treatments was administrated directly into the stomach through a gastric tube within a period of 2–3 min. Blood samples were drawn up to 48 h after dosing.

Mechanistic Studies

Based on the results from the meal effect studies, a cholecystokinin (CCK) A-receptor antagonist, devazepide (ML Laboratories PLC, Wavertree Technology Park, Liverpool L13 1EN, England), was utilized to block Intralipid®-stimulated biliary secretion in three dogs. Devazepide was dissolved in 1% (w/v) polysorbate 80, 70% (w/v) polyethylene glycol 400, and adjusted to final volume with 30% (v/v) alcohol to yield a final concentration of 2 mg/mL. Devazepide (0.1 mg/kg) was infused iv over a 5-min period prior to LY303366 coadministration with the 250 kcal/240 mL Intralipid® meal. This devazepide dose totally blocks oleate-stimulated gallbladder contraction and biliary secretion in dogs.[13]

Sample Analysis

Plasma concentrations of LY303366 were measured using HPLC–UV according to a method described previously.[14] Briefly, plasma samples were extracted on an IST 20-position manifold using a Bond Elut phenyl (PH) SPE column. The chromatographic conditions were as follows: column, Zorbax SB-C8 (4.6 × 250 mm, 5-μm particle size) analytical column with an in-line 2-μm filter; mobile phase, 45% acetonitrile and 55% 50 mM ammonium phosphate (pH 4.5) buffer; flow rate, 1.0 mL/min; temperature, 30 °C; and detection absorbance at 300 nm. LY306168 was used as the internal standard. The linear range of the assay was 20–5000 ng/mL. Based on analysis of control samples prepared at 20, 600, and 5,000 ng/mL, interday accuracy and precision were 91.1–114.1 and 0.6–4.0%, respectively. Intraday accuracy and precision were 90.2–99.5 and 5.1–6.3%, respectively. When stored at −70 °C, LY303366 in dog plasma is stable >5 months.

Data Statistical Analysis

A paired t test was used for all data treatment unless otherwise stated.

RESULTS

Formulation Effects in Fasted Dogs

Despite the positive influence of increased surface area (reduced particle size) on the *in vitro* dissolution rate of LY303366 capsules, as shown in Figure 2, such increase did not translate to an improvement in LY303366 systemic absorption in fasted dogs. Mean (±1 standard error (SE)) $AUC_{0-\infty}$ was 23.7 ± 3.64 μg · h/mL following administration of a 250-mg dose prepared using LY303366 bulk of 154 μm mean particle size. This result was not different ($p > 0.05$) than the value of 16.9 ± 2.67 μg · h/mL obtained following the same dose prepared using the bulk material with an average particle size of 11 μms. There was also no effect of particle size on C_{max}, T_{max} (time to reach C_{max}) and terminal half-life. These results suggest that dissolution does not represent the rate-determining step in the process of LY303366 systemic absorption.

Caco-2 Permeability

Figure 3 is a summary of LY303366 permeability following either apical or basolateral exposure of monolayers for 120 min. LY303366 permeability in the absorptive direction was independent of donor concentration and averaged 3.5 ± 1.10 × 10^{-6} cm/s ($n = 12$). The permeability of 100 μM mannitol in this direction was 1.9 ± 0.37 × 10^{-6} cm/s ($n = 12$), which was significantly less than

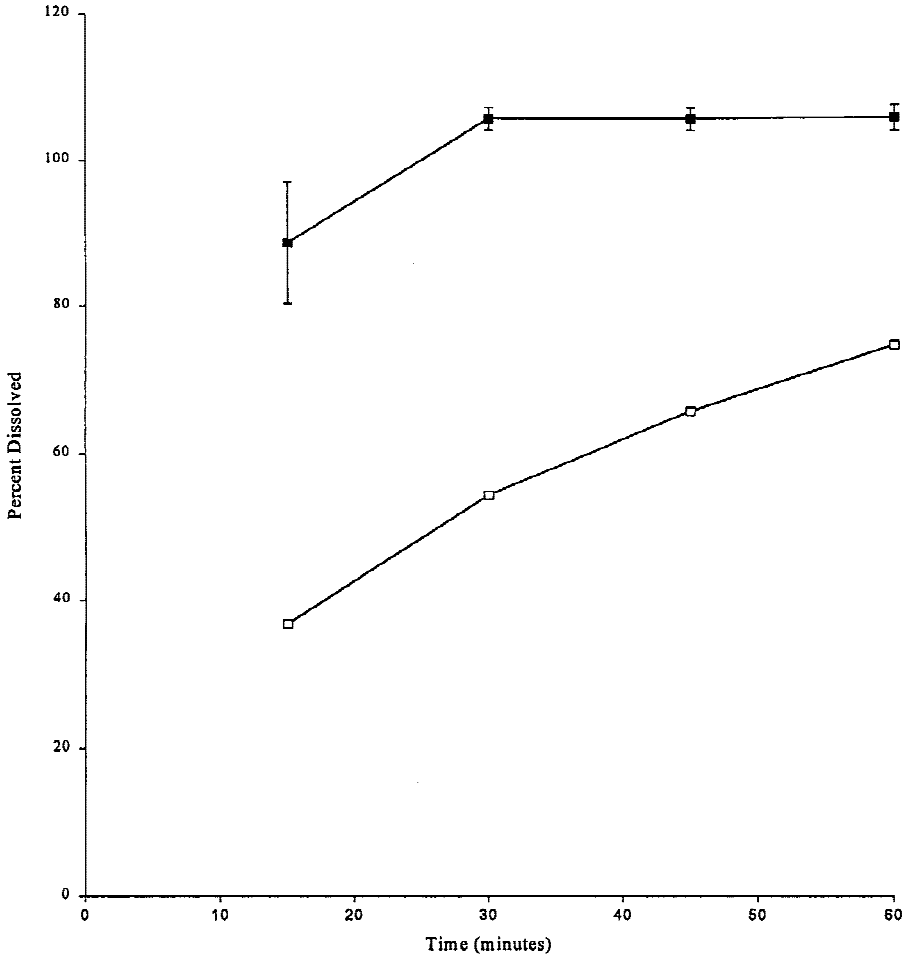

Figure 2. Dissolution profile of capsules containing 125 mg of LY303366 with an average particle size of either (■) 11 μm or (□) 154 μm. Dissolution testing was carried out in a standard Type 1 dissolution apparatus (100 rpm /paddle) using 900 mL of water containing 1% Tween 80. The two formulations were tested three times; error bars represent one SD.

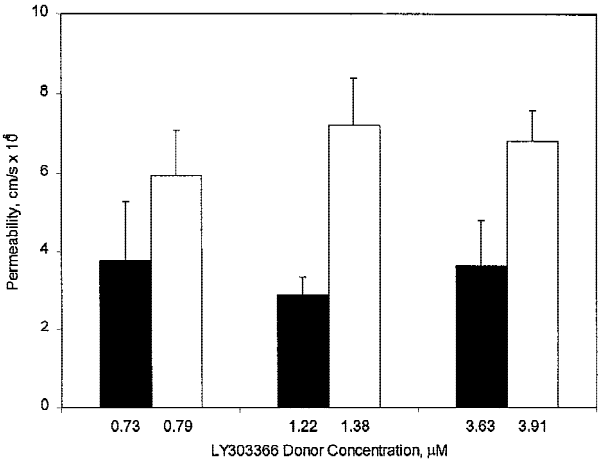

Figure 3. Ly303366 permeability coefficients following wither (■) apical or (□) basolateral exposure to three concentrations of LY303366. Values are mean ±SD; n = 3–6 filters for each concentration.

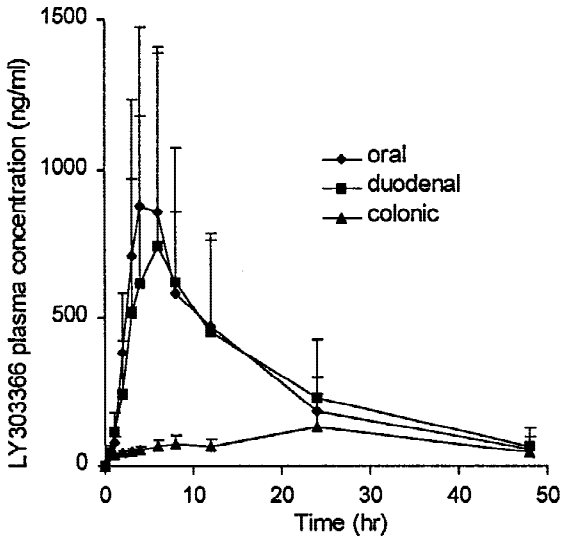

Figure 4. Mean ± SD plasma concentration profiles of LY303366 following oral and regional administration in three dogs.

LY303366 permeability (p = 0.0002). In the secretory direction, LY303366 permeability was also independent of concentration, but was ~2 times greater than permeability in the absorptive direction ($p < 0.0001$, one-way analysis of variance (ANOVA)), with an average permeability of $6.8 \pm 0.95 \times 10^{-6}$ cm/s. Mannitol permeability was 1.9 ±0.42 (n = 11) in the secretory direction. Although significantly different, mannitol and LY303366 absorptive permeabilities were only ~10–15% of dexamethasone permeability, 23.7 ± 0.60 $\times 10^{-6}$ cm/s, which is well absorbed (>75%) in humans. Both LY303366 and dexamethasone[15] have measured log octanol–water partition coefficients of 2.0.

At 120 min, cell-associated LY303366 was independent of donor concentration but accounted for 7.3 ± 1.21 and 13.5 ± 0.83% of the total amount of LY303366 initially present for the absorptive and secretory transport directions, respectively. In contrast, cell-associated mannitol was independent of transport direction and, at 0.08 ±0.020%, was substantially less than LY303366 binding.

Regional-Dependent Absorption

The extent of LY303366 absorption following duodenal administration was similar to that following oral administration. However, significantly lower drug plasma levels were obtained following administration through the colonic access port (Figure 4). Both AUC $_{0-48h}$ and C_{max} from colonic administration were significantly decreased ($p < 0.05$) when compared with oral fasted administration of LY303366. Administration of LY303366 through a mid-jejunal intestinal access port in one dog resulted in a substantial decrease in drug plasma levels compared with oral and duodenal administration. Regional AUC_{0-48h} values were 23.3, 26.5, 17.7, and 6.9 μg · h/mL and C_{max} 1.6, 1.5, 0.6, and 0.3 μg/mL from oral, duodenal, jejunal, and colonic administration, respectively, in this dog.

Meal Effect Studies

The contributions of meal osmolality versus caloric density to reduction in drug plasma levels were studied by comparing the influence of a 480-mOsm/kg noncaloric saline meal with a control saline (290 mOsm/kg) drug administration and a mixed meal (480 mOsm/kg, 250 kcal) treatment. As shown in Figure 5, absorption of LY303366 was not affected by noncaloric meal hyperosmolality (p = 0.35). A significant decrease in drug plasma levels was observed with coadministration of the 250-kcal, hyperosmolar liquid nutrient meal of mixed caloric composition. Furthermore, both an Intralipid® meal of equivalent caloric density and a reduced calorie protein meal (125 kcal) also resulted in depressed LY303366 plasma levels compared with drug coadministration with saline in fasted dogs (Figure 6, Table 1).

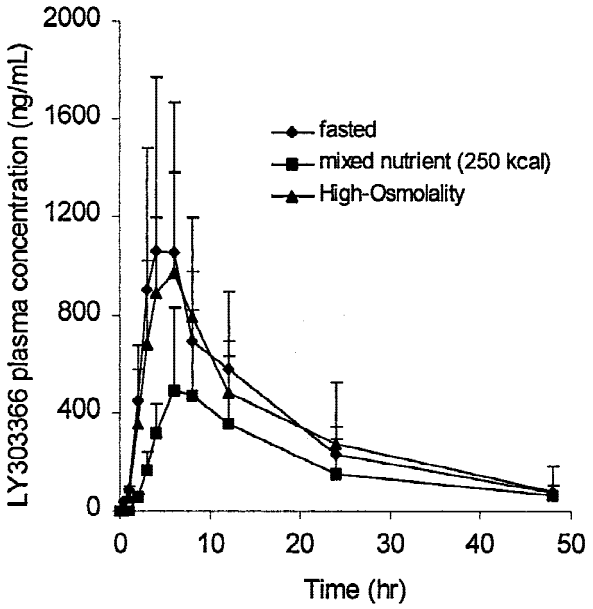

Figure 5. Mean ± SD plasma concentration profiles of LY303366 after oral administration to fasted dogs, or following LY303366 coadministration with either a mixed liquid nutrient or a noncaloric high osmolality NaCl solution.

In contrast to the negative meal effect observed with either a mixed meal, a lipid meal, or a protein meal, coadministration of LY303366 with a 125-kcal carbohydrate (either sucrose or Moducal®) meal did not decrease drug plasma levels (Figure 6). In fact, a trend toward increasing LY303366 levels was indicated by the AUC and C_{max} data, although these values were not significantly different than controls. It is also noteworthy that meal treatments did not increase LY303366 plasma level T_{max}, which averaged 6.0 h for all treatments including the fasted-state controls.

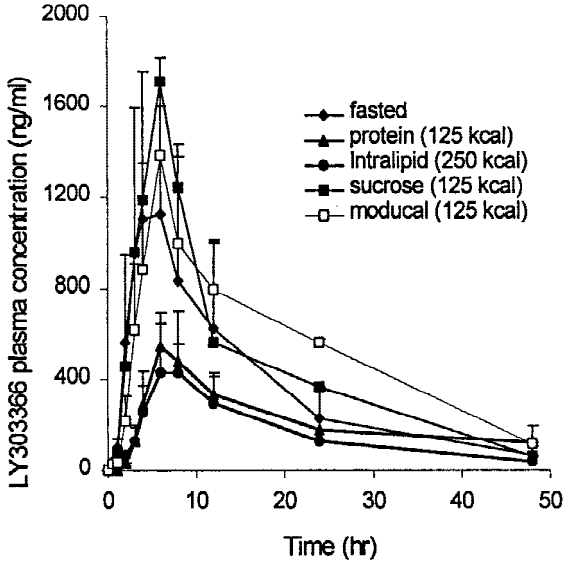

Figure 6. Mean ± SD plasma concentration profiles of LY303366 after oral administration to fasted dogs, or following LY303366 coadministration with Intralipid®, protein, or carbohydrate (sucrose and Moducal®) meals.

Mechanistic Study

As shown in Figure 7, pretreatment of three dogs with devazepide failed to reverse the reduction of LY303366 plasma levels observed with Intralipid®.

DISCUSSION

LY303366 possesses a low aqueous solubility and a high octanol–water partition coefficient. However, an increase in dissolution rate (Figure 2) did not lead to an increase in systemic absorption. Furthermore, lipophilic drugs typically provide for good colonic absorption as demonstrated in a canine colonic absorption model.[16] This behavior is not observed with LY303366 (Figure 4) in the same canine colonic access port model. These *in vitro* and *in vivo* studies suggest that the intestinal absorption of LY303366 is not strictly dissolution-rate limited.

The low LY303366 absorptive permeability in human intestinal cell monolayers suggested that *in vivo* absorption of LY303366 might be limited by membrane permeability. Whereas LY303366 has a lipophilic side chain, the polyhydroxyl structure of the ECB nucleus promotes hydrogen bonding and likely limits membrane permeation.[17] If permeability plays a role in limiting the absorption of LY303366, it is not surprising that exposure of the drug to less absorbing surface area with jejunal and colonic administration provides lower plasma levels than when the drug sees more absorbing intestinal surface with oral or duodenal administration (Figure 4).

As shown in directional transport studies in human colon cell monolayers (Figure 3), intestinal secretion, possibly mediated by P-glycoprotein, was observed. Secretory flux of LY303366 was twofold higher compared with absorptive flux. It has been reported that antifungal resistance to ECB analogs is partially mediated by

Table 1. Comparison of Effects of Meal Treatments on LY303366 Plasma Level Pharmacokinetic Parameters following Oral Administration of a 250-mg Dose to Dogs

Parameter	Fasted	Mixed Meal	Lipid Meal	Protein Meal	Carbohydrate Meals
AUC_{0-48} (μg · h/mL)	21.2 ± 5.84	8.9 ± 2.56*	7.5 ± 1.84*	8.9 ± 2.77*	25.2 ± 5.13
C_{max} (μg/mL)	1.1 ± 0.27	0.5 ± 0.17*	0.4 ± 0.13*	0.5 ± 0.20*	1.6 ± 0.30

[a] Results are expressed as mean ± SE; T_{max} averaged 6.0 h for all treatments; administered volumes are five times higher than those used in the particle size study; *paired *t*-test *p* value ≤ 0.05 relative to fasted conditions.

membrane proteins encoded by MDR-like genes.[18] However, the contribution of an intestinal region-specific drug secretion effect to the observed regional dependence in LY303366 absorption is unknown because of the multiplicity of secretory transporters and lack of understanding regarding their distribution in this tissue. In addition to demonstrating a net secretion of LY303366, Caco-2 experiments also revealed substantial binding of LY303366 to the human colon cell monolayers. In fact, the percent bound is in the range of the oral bioavailability. This extensive binding might be expected to prolong intestinal residence time and drug availability for absorption, which might, in turn, contribute the prolonged oral absorption of LY303366 (T_{max}, 6 h). Poor and prolonged absorption behavior has also been reported for the antifungal, amphotericin B, a drug with high hydrogen bonding potential and oral bioavailability and T_{max} similar to that of LY303366.[19]

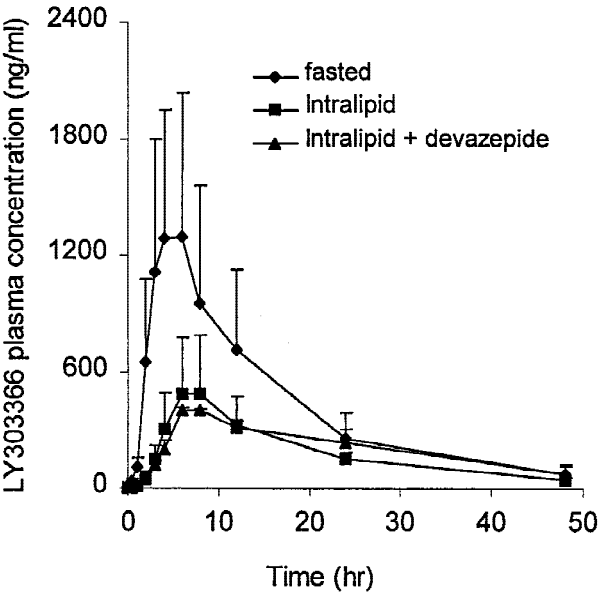

Figure 7. Mean ± SD plasma concentration profiles of LY303366 after oral administration to fasted dogs, or following LY303366 coadministration with either Intralipid® or Intralipid® with devazepide pretreatment.

In a preliminary canine study, a single 5-mg/kg oral administration of LY303366 provided drug plasma levels above the MIC for *C. albicans* over a 20-h period and for *Aspergillus fumigatus* over a 10-h period. However, when the same dose was coadministered with a solid dog food meal, drug plasma levels dropped below the MIC for *A. fumigatus* (data not shown). In a follow-up evaluation conducted in dogs, a 30% decline in both $AUC_{0-\infty}$ and C_{max} was observed when a 250-mg dose of LY303366 was coadministrated with a solid meal.[20] As part of an oral, single-dose clinical trial evaluation of LY303366 safety, a negative food effect was also observed. Specifically, concurrent administration of a 50-mg dose with a high-fat meal resulted in a 75% reduction in $AUC_{0-\infty}$. Coadministration of a 700-mg dose with a low-fat meal resulted in a 35% reduction in $AUC_{0-\infty}$.[4] In this study, the influence of meal volume and composition was further explored in dogs.

A negative meal effect on drug plasma levels can be generated when the volume between fasted- and fed-state treatments is not kept constant. It is often the case in meal effect studies that only a small volume of fluid is administered when drug is given in the fasted state, whereas a large meal volume is given with the drug in fed-state study treatments. The larger volume serves to dilute the drug concentration exposed to absorbing membranes as well as to alter the gastric emptying time of drug into the small intestine.[25] Only 25 mL of water was orally coadministered with the drug in the regional-dependence studies, 50 mL in the drug particle size studies, and 240 mL of water or saline in the meal effect control studies. The drug plasma level data in Table 1 and Figures 4, 5, and 6 suggest that a dilution effect resulting from differences in coadministered volume does not influence drug plasma lev-

els in the fasted state to account for meal effect observations.

Because the drug does show some instability at acidic pH, the fact that a meal would prolong gastric residence time might also be considered to play a role in a negative meal effect. However, similar drug plasma levels are observed following oral and duodenal administration. Oral drug administration with a noncaloric hyperosmolar solution, which also prolongs gastric residence time compared with iso-osmolar solutions, also did not lead to plasma level reduction and lends further support to the conclusion that acid instability is not a limiting factor in LY303366 absorption.

In previous clinical studies,[4] it was observed that a negative meal effect could be diminished by reducing caloric and fat content and by increasing the drug dose. In the canine meal composition studies, both lipid and protein meals with different caloric density resulted in a similar decrease in LY303366 absorption (Figure 6). In contrast, carbohydrate meals did not cause negative meal effects but rather showed a trend toward increasing drug plasma levels, though this effect was not statistically significant (Table 1).

Another possible cause of the observed negative food effect is the influence of meal viscosity and/or drug binding to meal components. These factors are particularly important when a significant fraction of the drug is absorbed in the upper intestine, as is the case with LY303366 (Figure 4). Under fed-state conditions, meal viscosity and drug binding potential is greatest in the upper intestine where digestion is at its least complete stage and will serve to reduce the amount of drug that gets to the intestinal absorbing surface. In previous studies in the same dog model with another compound, bidisomide, solid meals were shown to produce a significant negative meal effect on drug plasma levels, whereas equivalent caloric liquid meals of relatively low viscosity did not. The negative meal effect on bidisomide, which also showed better absorption in the upper than lower intestine, was generated by viscous zero calorie meals.[22] Bidisomide binding to meal components was shown not to underlie the negative meal effect in equilibrium dialysis experiments.

In a preliminary study, LY303366 was coadministrated with a solid meal, and drug absorption was observed to decrease by 30% compared with fasted control studies.[5] However, the depression of LY303366 plasma levels observed upon its coadministration with a mixed composition liquid meal of low viscosity (Figure 5) was equivalent to that observed with the solid meal coadministration. This result suggests that meal viscosity is not a significant factor in reducing the absorption of LY303366. The possibility that LY303366 binds to Intralipid® and Casec® but not to sucrose or Moducal® has not been ruled out in this study. Because the drug is a lipopeptide, such a selective interaction is certainly a possibility and could underlie the negative food effect in the case of lipid and protein. However, on the basis of hydrogen binding potential, it might be projected that LY303366 would interact most readily with sugars and polysaccharides.

Another consideration for the difference in effects on LY303366 absorption as a function of meal composition is that fat and protein meals stimulate biliary secretion whereas carbohydrates do not. Bile-salt-mediated micellar solubilization of poorly water-soluble compounds may decrease the intermicellar "free" fraction of drug in the upper intestine, which in turn could lead to a decrease in drug absorption.[23] *In vitro* studies have shown that devazepide is an effective inhibitor of CCK binding to CCK-A receptors in the pancreas (IC_{50}, 81 pM) and gallbladder (IC_{50}, 45 pM).[11] In a study originally carried out at Merck, Sharp and Dohme Research Laboratories, it was observed that devazepide completely blocked oleate-stimulated biliary secretion in bile-duct cannulated dogs.[24] Human studies have also shown that 10 mg of devazepide is orally active, and completely inhibits meal-induced gallbladder contraction with no effect on gastric-emptying.[25] Previous studies in our laboratory have shown that blockade of lipid-stimulated biliary secretion with devazepide pretreatment nullified a meal effect in connection with the absorption of phenytoin.[26] However, in the present study, pretreatment with devazepide failed to reverse the negative meal effect when LY303366 was coadministrated with Intralipid® (Figure 7). Based on these results, it appears that biliary secretion is not likely to be responsible for the observed negative influence of lipid- and protein-containing meals on LY303366 absorption. In contrast, lipid–bile salt mixed micelles have been shown to enhance the gastrointestinal absorption of amphotericin-B.[27]

In summary, although initial physicochemical LY303366 data suggested that low oral bioavailability might be the result of low aqueous solubility and a dissolution-rate limit to drug absorption, drug formulation and intestinal permeability studies point toward a permeability limitation

for absorption. Thus, LY303366 might be regarded as a Class IV (low solubility — low permeability) drug in accordance with the Biopharmaceutical Classification System.[28] Coadministration of meals with LY303366 leads to a reduction in drug plasma levels in both dogs and humans compared with administration without food. The meal effect is composition dependent in that lipid and protein meals decrease drug plasma levels whereas carbohydrate meals do not. Caco-2 experiments suggest that the prolonged absorption profile for LY303366 may be the result of drug adherence to intestinal membranes. However, there is no direct evidence for this suggestion, and the mechanism underlying the negative meal effect has not been resolved at this time.

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