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Effect of Uptake Transporters OAT3 and OATP1B1 and Efflux Transporter MRP2 on the Pharmacokinetics of Eluxadoline

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Abstract

The effects of OATP1B1, OAT3, and MRP2 on the pharmacokinetics of eluxadoline, an oral, locally active, opioid receptor agonist/antagonist being developed for treatment of IBS-d were assessed in vivo. Coadministration of a single 200 mg dose of eluxadoline with cyclosporine, and probenecid increased eluxadoline systemic exposure [$AUC_{(0-inf)}$] by 4.4- and 1.4-fold, respectively, whereas peak exposure (C_{max}) increased 6.2-fold and 1.3-fold, respectively. Cyclosporine had little effect on renal clearance (CL_{ren}) of eluxadoline whereas probenecid reduced CL_{ren} by nearly 50%. These study results suggested that sinusoidal OATP1B1-mediated hepatic uptake of eluxadoline (during first-pass and systemic extraction) plays a major role in its absorption and disposition, whereas OAT3-mediated basolateral uptake in the proximal renal tubules and MRP2-mediated canalicular and renal tubular apical efflux play only minor roles in its overall disposition. All treatments were safe and well tolerated.

Keywords

eluxadoline, pharmacokinetics, drug interaction, cyclosporine, probenecid

Eluxadoline is a locally active, mixed μ-opioid receptor (μOR) agonist and δ-opioid receptor (δOR) antagonist[1] being developed for the treatment of IBS-d (diarrhea-predominant irritable bowel syndrome).[2,3] Eluxadoline has gastrointestinal (GI) transit-inhibiting activity that is consistent with its primary pharmacological profile as a local μOR agonist; however, its additional δOR antagonist activity mitigates the profound constipating effect observed with unopposed peripherally acting μOR agonists (eg, loperamide or diphenoxylate).[4] Based on results of absolute oral bioavailability, ^{14}C-labeled mass balance, and hepatic portal and jugular vein concentration studies in animals (FK10138, FK5756, and DD07389 on file at Furiex), eluxadoline is poorly orally absorbed and undergoes moderate hepatic first-pass extraction with biliary excretion in rats and dogs. Following administration of a single 300 mg dose of radiolabeled eluxadoline to humans, an average of 0.12% (0.00%–0.42%, n = 6) of the administered dose was recovered in urine after 192 hours, and 82% (50%–105%) of the administered dose was recovered in feces after 336 hours. Additionally, approximately 90% or more of the administered dose was recovered in the feces in 4 of 8 subjects while no circulating metabolites were detected (data on file at Furiex).

In vitro and in vivo studies have demonstrated the following (data on file at Furiex): the absence of hepatic drug metabolism (either in vitro or in vivo with the exception of slow formation of a glucuronide metabolite found in human urine after a 1,000 mg oral dose only), high GI solubility, and poor Caco2 cell-line permeability, the latter most likely a result of the zwitterionic nature of eluxadoline (see[5] for description of molecular structure). In in vitro studies, eluxadoline was found not to be transported by OAT1, OCT1, OCT2, OATP1B3, P-gp, or BCRP, but was transported by OAT3, OATP1B1, and BSEP at the highest concentration tested (ie, 400 ng/mL, which is 162-fold larger than observed C_{max} of the highest therapeutic dose of 100 mg). MRP2-dependent vesicular accumulation of eluxadoline was observed, indicating eluxadoline was a substrate of MRP2 under the experimental conditions. Eluxadoline did not inhibit BCRP-, BSEP-, MRP2-, OCT1-, OCT2-, OAT1-, OAT3-, OATP1B3-mediated transport of probe substrates, but did inhibit the transport of probe substrates of OATP1B1 and

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P-gp with respective inhibition of 32.6% and 6.25% (at concentrations approximately 162-fold higher than observed plasma concentrations at the maximum therapeutic dose of 100 mg). Finally, no inhibition or induction of CYP-450s was observed in in vitro studies.

Based on these findings, we concluded that drug-drug interactions (DDIs) involving CYP450s were unlikely. However, the in vitro drug transporter studies suggested eluxadoline could be an in vivo substrate of MRP2, OAT3, and OATP1B1 and an inhibitor of OATP1B1, depending on the concentration of drugs at the relevant physiological sites. Therefore, we designed a prospective in vivo DDI study with cyclosporine as prototypical OATP1B1 and MRP2 inhibitor[6–10] and probenecid, a prototypical MRP2 and OAT3 inhibitor,[6,10] in order to investigate the clinical relevance of any involvement of MRP2, OAT3, and OATP1B1 in the absorption and disposition of eluxadoline.

Methods

The study was conducted in accordance with all relevant federal guidelines and institutional policies including, but not limited to, informed patient consent prior to enrollment and prior review and approval of the study protocol and informed consent form.

The study was designed as an open-label, single-center, randomized-sequence, three-treatment/period crossover study in order to evaluate the effects of cyclosporine and probenecid on the pharmacokinetics of eluxadoline and the safety and tolerability of single oral doses of eluxadoline administered alone and in combination with cyclosporine and probenecid in healthy volunteers.

Thirty healthy male and female volunteers were randomized to receive 1 of 6 treatment sequences with Treatment A: single 100 mg dose of eluxadoline alone; Treatment B: single 100 mg dose of eluxadoline coadministered with a single 600 mg dose of cyclosporine and; Treatment C: single 100 mg dose of eluxadoline coadministered with a single 500 mg dose of probenecid. For each subject, the study consisted of 3 phases: a screening phase (up to 28 days before Day 1 of Period 1), a treatment phase (3 treatment periods), and a posttreatment phase (end-of-study or early withdrawal visit). Treatment periods were separated by 7-day washout periods. The total duration of study participation for each subject was approximately 8 weeks. Eligible subjects were admitted to the clinical research unit (CRU) on Day –1 of each period, underwent scheduled procedures, and a 10 hour overnight fast from food. All subjects refrained from drinking water for at least 1 hour before dosing. Subjects were discharged from the CRU on Day 4 of each period after completion of all scheduled procedures.

Blood samples for plasma PK analysis were collected predose (within 45 minutes before dosing), and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 24, 48, and 72 hours after administration of eluxadoline. Urine samples were collected predose (immediately before dosing) and, after dosing, at intervals of 0–4, 4–8, 8–12, 12–24, 24–48, and 48–72 hours.

Quantitation of eluxadoline concentrations in plasma and urine samples was conducted by PPD Bioanalytical Laboratories, Richmond, Virginia, using validated, specific, and sensitive liquid chromatography-tandem mass spectrometry methods.

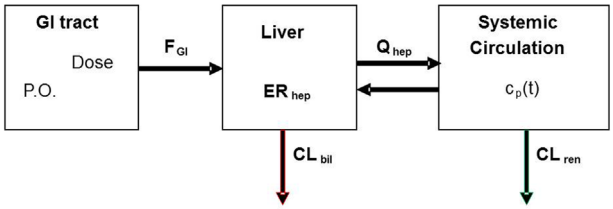
The lower/upper limits of quantitation (LLQ/ULQ) in human plasma were 0.100 and 100 ng/mL, respectively. The assays were linear ($R^2 \geq 0.990$) over the calibration range. Each run included 5 levels of quality control (QC) samples assayed in duplicate that spanned the calibration range. Interassay precision, estimated as percent coefficient of variation (CV) among sets of QC samples from each run, ranged from 3.5% to 5.4%. Interassay accuracy, estimated as the percent difference from nominal concentration among sets of QC samples from each run, ranged from −2.7% to 3.7%.

The LLQ/ULQ in human urine were 1.00 and 1,000 ng/mL, respectively. Assays were linear ($R^2 \geq 0.990$) over the entire calibration range. Each run included 5 levels of QC samples assayed in duplicate that spanned the calibration range. Interassay precision ranged from 2.1% to 5.5%. Interassay accuracy ranged from −7.85% to 3.87%.

Individual plasma concentration vs. actual time profiles for eluxadoline were used to derive pertinent exposure/PK parameters by noncompartmental analysis (NCA) using WinNonlin® (Phoenix) Version 6.2 (Pharsight Corporation, St Louis, Missouri). Urinary concentrations for each collection interval were multiplied by the corresponding urine volumes to determine urinary excretion; cumulative urinary excretion over 72 hours as fraction of the dose ($\%Fe_{(0-72)}$) was determined, and renal clearance (CL_{ren}) was estimated by $\%Fe_{(0-72)}$*Dose/AUC_{0-inf}.

As human IV PK information is not available, CL_{TOT} of eluxadoline was predicted by allometric PK scaling performed by linear regression of CL_{TOT} onto body weight (BW) as $\ln(CL_{TOT}) = \ln(BW)$.[11] A schematic of an integrated, semiphysiological PK/ADME model along with equations used for the model are shown in Figure 1. This model was used to quantitatively simulate the effects on eluxadoline exposure and CL_{ren} due to the various interventions by adjusting the biologically relevant ADME properties (CL_{int} and CL_{TS}) of eluxadoline. The PK/ADME parameter descriptions are displayed in Table 1. Changes (geometric mean ratios) in $AUC_{(0-inf)}$ and CL_{ren} were used to assess the impact on PK/ADME.

Each of the PK parameters is reported descriptively as (arithmetic) mean and coefficient of variation (CV), except T_{max}, where median and min, max are tabulated (Table 2). Estimation of the elimination rate constant



$$CL_{TOT} = CL_{bil} + CL_{ren} \quad Eq.1$$

$$CL_{bil} = ER_{hrp} * Q_{hep} \quad Eq.2$$

$$F_{oral} = F_{GI} * \left(1 - ER_{hep}\right) \quad Eq.3$$

$$ER_{hep} = f_u * CL_{int} / \left(f_u * CL_{int} + Q_{hep}\right) \quad Eq\ 4.$$

$$CL_{ren} = GFR * f_u + CL_{TS} \quad Eq.5$$

Figure 1. Semiphysiological integrated PK/ADME model schematic and equations.

required: at least 3 points in the terminal phase, duration of time in the terminal phase was at least 3 times the estimated half-life, and the extrapolation had to be less than 20%. For formal statistical assessment of a DDI effect on the single-dose pharmacokinetics of eluxadoline, analysis of variance (ANOVA) was performed using the natural log-transformed exposure metrics ($AUC_{[0–inf]}$ and C_{max}). The ANOVA model included sequence, period, and treatment as fixed effects and subject within sequence as a random effect. Treatment A (ie, eluxadoline, 100 mg single dose) was defined as the reference treatment, and Treatment B (eluxadoline, 100 mg single dose and cyclosporine, 600 mg single dose) and Treatment C (eluxadoline, 100 mg single dose, and probenecid, 500 mg single dose) were defined as the test treatments. Least squares (LS) means and difference of LS means for the log-transformed $AUC_{(0–inf)}$ and C_{max} were back-transformed to obtain the geometric means and ratios of geometric means on the original scale (B/A and C/A), respectively. The 90%-confidence intervals (CIs) for the ratio of the geometric mean are also reported. The ANOVA was performed using 2 approaches: the first used only subjects that had an estimable elimination rate for all 3 treatments given (n = 7) and the second used all concentration time profiles with estimable elimination rates (n = 20, n = 19, and n = 20 for treatments A, B, and C, respectively). A significant DDI was concluded when the 90%CIs of the ratios (B/A and C/A) for both $AUC_{(0–inf)}$ and C_{max} were not included within the range of 0.80–1.25. All statistical analyses were performed using SAS (SAS Institute Inc., Cary, North Carolina) version 9.2.

To assess the DDI effects of cyclosporine and probenecid, relevant ADME model parameters, namely CL_{int}, and CL_{TS}, were adjusted to match the observed increases in $AUC_{(0–inf)}$ (predicted as F_{oral}*Dose/CL_{TOT}) and observed reduction in CL_{ren}.

In an attempt to separate the effects of OATP1B1 on F_{oral} vs. systemic biliary clearance (CL_{bil}) and to estimate the contribution of CL_{ren} vs. CL_{bil} to overall CL_{TOT}, we used allometric PK scaling to estimate human CL_{TOT} and, subsequently, F_{oral}. The equations shown in Figure 1 were also used to estimate other ADME properties. CL_{bil} was estimated, using equation 1, as the difference between CL_{TOT} and (observed) CL_{ren}, whereas hepatic extraction (ER_{hep}) was estimated from equation 2 as CL_{bil}/Q_{hep}. F_{oral} was considered the product of the fraction absorbed across the GI wall (F_{GI}) and 1-ER_{hep}, equation 3. Finally, hepatic intrinsic (biliary) clearance (CL_{int}) was estimated using the Wilkinson-Shand (hepatic venous equilibrium model) equation 4, whereas (net) tubular secretion clearance (CL_{TS}) was estimated from equation 5 as CL_{ren}/f_u-GFR.

All adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA®), Version 11.0. Data collected for safety evaluations included clinical safety labs, physical exams, vital signs, and 12-lead electrocardiograms. Finally, ancillary data such as medical history (coded using MedDRA®, Version 11.0) and prior and any concomitant medications (coded using WHO Drug Dictionary Enhanced 01, Dec 2009) were collected and reviewed.

Results

The average age of subjects was 31 years and ranged from 20 to 48 years. Seventy percent of the subjects (n = 21) were male; the mean (SD) weight was 75.9 kg (12.5); 20 subjects were white, whereas 10 were African American. For illustrative purposes, mean (±SD) eluxadoline plasma concentrations vs. time are presented on a semilogarithmic scale in Figure 2. Mean eluxadoline plasma concentrations were quantifiable

Table 1. PK/ADME Parameter Definitions

F_{oral}	Oral bioavailability	CL_{TS}	Tubular secretion clearance
F_{GI}	Extent of GI absorption/GI permeability	ER_{hep}	Hepatic extraction ratio
CL_{TOT}	Total clearance	CL_{bili}	Biliary clearance
CL_{ren}	Renal clearance	Q_{hep}	Hepatic blood flow (1,500 mL/min)
CL_{int}	Intrinsic biliary clearance	f_u	Unbound fraction of drug in plasma

Table 2. Summary Statistics and Statistical Analysis of Plasma Pharmacokinetic Parameters for Eluxadoline

Arithmetic Mean (CV) Plasma and Urine Pharmacokinetic Parameters of Eluxadoline

	Eluxadoline 100 mg (N = 29)	Eluxadoline 100 mg Plus Cyclosporine 600 mg (N = 30)	Eluxadoline 100 mg Plus Probenecid 500 mg (N = 29)
C_{max} (ng/mL)	3.1 (92)	20.9 (85)	3.6 (59)
$AUC_{(0–inf)}$ (ng·hr/mL)[a]	18.0 (67)	75.3 (62)	23.0 (56)
T_{max} (hours)[b]	2.1 (0.25, 6.0)	2.5 (1.5, 4.0)	2.5 (0.25, 6.0)
$t_{1/2}$ (hours)[a]	3.7 (54)	7.4 (80)	5.1 (77)
CL/F (L/h)[a]	7550 (54)	1943 (69)	5645 (53)
Vz/F (L)[a]	39318 (82)	20728 (88)	37145 (87)
$\%Fe_{(0–72)}$	0.12 (52)	0.46 (61)	0.08 (39)
CLr (L/h)	7.0 (20)	5.8 (20.0)	3.7 (25)

Statistical Analysis of Plasma Exposure Metrics for Eluxadoline

Parameter (unit)	Treatment[c]	N	Geometric LS Means	Treatment Comparison	Ratio (%) of Geometric LS Means	90%CI of the Ratio
C_{max} (ng/mL)	A	29	2.4	–	–	–
	B	29	14.7	B/A	6.18	(5.14, 7.44)
	C	29	3.1	C/A	1.31	(1.09, 1.58)
$AUC_{(0–inf)}$ (ng·hr/mL) [d]	A	7	15.7	–	–	–
	B	7	79.9	B/A	5.09	(3.81, 6.81)
	C	7	20.4	C/A	1.30	(0.97, 1.74)
$AUC_{(0–inf)}$ (ng·hr/mL) [e]	A	20	14.7	–	–	–
	B	19	62.6	B/A	4.25	(3.52, 5.13)
	C	20	21.8	C/A	1.48	(1.24, 1.78)

CV, coefficient of variation; h, hours; L, liters; CI, 90%-confidence interval; LS, least squares.

[a]n = 20 for eluxadoline alone; n = 19 for eluxadoline + cyclosporine; n = 20 for eluxadoline + probenecid.

[b]For T_{max}, the median (minimum, maximum) values are presented.

[c]Treatment A = single 100 mg dose of eluxadoline. Treatment B = single 100 mg dose of eluxadoline + single 600 mg dose of cyclosporine. Treatment C = single 100 mg dose of eluxadoline + single 500 mg dose of probenecid.

[d]Requires that a subject has estimable elimination rates for all 3 treatments to be included in the ANOVA model.

[e]All concentration time profiles with estimable elimination rates included in the ANOVA model.

through 72 hours after administration for eluxadoline alone and eluxadoline with cyclosporine, but were quantifiable through 48 hours only for eluxadoline with probenecid.

Cyclosporine coadministration resulted in a consistent elevation of eluxadoline plasma concentrations throughout the entire 72-hour sampling period, whereas probenecid coadministration led to a small and transient increase in mean plasma concentrations only. On average, the terminal decline of eluxadoline in the presence of either cyclosporine or probenecid appears similar as shown in Figure 2.

The NCA-derived PK parameters and exposure metrics are tabulated in Table 2. Overall, the $AUC_{(0–inf)}$ is well estimated as the portion extrapolated was less than 20% in subjects with estimable half-lives. The terminal rate constant was estimable for: 20 (70%) eluxadoline profiles, 19 (63%) eluxadoline + cyclosporine profiles, and 20 (70%) eluxadoline + probenecid profiles (Table 2). The maximum concentrations for eluxadoline at the later sampling timepoints were close to the LLQ: at 48 and 72 hours, mean eluxadoline concentrations were 0.16 and 0.18 ng/mL, respectively; for eluxadoline + cyclosporine at 48 and 72 hours mean concentrations were 0.38 and 0.59 ng/mL, respectively; and for eluxadoline + probenecid at 48 and 72 hours mean concentrations were 0.11 and 0.0 ng/mL, respectively. The mean (standard deviation) of concentrations at 0.25 and 0.5 hours for eluxadoline alone were 0.45 (0.52) and 0.98 (0.72); 0.45 (0.33) and 1.21 (0.88) for eluxadoline + cyclosporine; and 0.98 (1.13) and 1.5 (0.97) for eluxadoline + probenecid.

Total systemic exposure ($AUC_{[0–inf]}$) and C_{max} values of eluxadoline were consistently elevated for eluxadoline with cyclosporine and eluxadoline with probenecid compared to eluxadoline alone.

The effects of cyclosporine on total eluxadoline exposures were much larger than the effects of probenecid. Median T_{max} values of eluxadoline were similar among the treatment groups whereas the increases in C_{max} corresponded to the elevations in total exposures. Mean (CV) $t_{1/2}$ values were higher for eluxadoline with cyclosporine (7.4 [80%] hours) and eluxadoline with

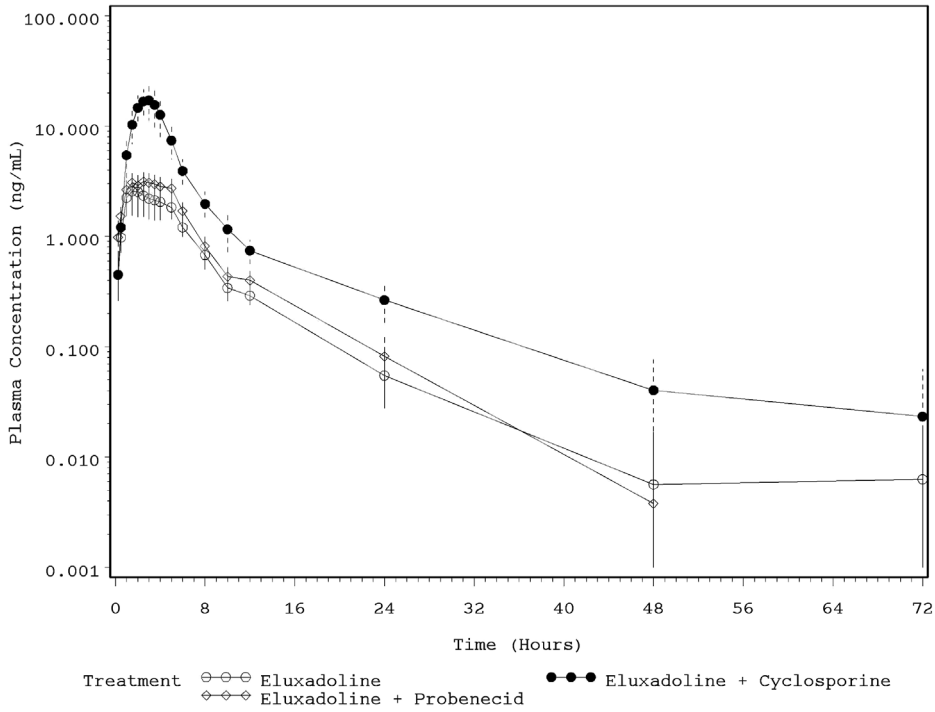


Figure 2. Mean (±SD) plasma concentrations of eluxadoline versus time.

probenecid (5.1 [77%] hours) compared to eluxadoline alone (3.7 [54%] hours).

Overall, the fraction of the eluxadoline dose excreted in urine unchanged (%Fe) was less than 1%, with or without cyclosporine and probenecid, whereas %Fe was increased in the presence of cyclosporine but decreased in the presence of probenecid. Mean (CV%) CL_{ren} for eluxadoline alone was estimated as 116 (20%) mL/min. In the presence of cyclosporine and probenecid, CL_{ren} values were reduced to 97 (19%) and 62 (25%) mL/min, respectively.

The results of the ANOVA on the eluxadoline exposure metrics are presented in Table 2 and statistically validate the observations above. The observed increases in C_{max} and $AUC_{(0–inf)}$ of eluxadoline coadministered with cyclosporine vs. eluxadoline alone, based on the geometric mean ratios, were 6.2 and 5.1-fold, respectively, whereas the increases due to probenecid were only 1.31 and 1.30-fold. The 90% confidence intervals for the geometric mean ratios of C_{max} and $AUC_{(0–inf)}$ of eluxadoline were outside the predefined target range of 0.80–1.25 for cyclosporine. Overall, cyclosporine had a large (6.2-fold) impact on peak and a slightly less (4.4-fold) impact on total exposure, whereas probenecid showed a mild and consistent (1.3-fold) effect on peak and total exposure. The ANOVA model leads to similar results irrespective of whether one uses only subjects with estimable elimination rates for all 3 treatments or uses all concentration time profiles with estimable terminal rate constants.

Using the available animal CL_{TOT} values—as shown in Table 3—for allometric PK scaling resulted in a linear regression equation of $\ln(CL_{TOT}) = 3.475 + 0.7830 \ln(BW)$ with an $R^2 = 0.98$. Back-transforming gives $CL_{TOT} = 32.30BW^{0.7830}$ resulting in an estimate for human CL_{TOT} (assuming a body weight of 75 kg) of 949 mL/min.

Table 3. PK Parameters From Animal Studies and Human Estimates From Allometric Scaling

Species	IV Dose (mg/kg)	BW (kg)	CL_{tot} (mL/min/kg)	$t_{½}$ (hour)	CL_{tot} (mL/min)
Rat	10	0.25	44.4	0.8	11.1
Cynos	10	4.3	18.54	3.73	77.9
Rhesus	3.2	5.41	28.5	0.65	154.2
Dog	2	8.6	19.92	0.75	171.3
Human	–	75	*12.6*		*949*

BW, body weight; cynos, cynomolgus monkeys.

Source: Furiex Studies DD7393, FK10138, FK10141, and FK10142.

Thus, for the reference case (without inhibitor), human F_{oral} for eluxadoline was estimated to be 1.02% whereas hepatic extraction ratio and F_{GI} were estimated as 55.8% and 2.3%, respectively. The observed mean $AUC_{(0–inf)}$ for eluxadoline was 18.0 ng·hr/mL, and PK-ADME-model-predicted value was 17.8 ng·hr/mL. For the two inhibitor scenarios (cyclosporine and probenecid), the observed mean $AUC_{(0–inf)}$ for eluxadoline was 75.3 and 23.0 ng·hr/mL, respectively, and the corresponding predicted values from the PK-ADME model, after optimizing the respective reductions in CL_{int} and CL_{TS} (see below), were 78.3 and 23.6 ng·hr/mL, respectively. The model-predicted CL_{ren} values of 92 and 62 mL/min, for cyclosporine and probenecid, respectively, were also quite similar to their observed counterparts of 92 and 58 mL/min, validating the model and final model parameter estimates.

Overall, 8 subjects (27%) reported a total of 21 adverse events (AEs) in the DDI study. The highest percentage of subjects (6 subjects, 20%) reported AEs after receiving eluxadoline in combination with cyclosporine, and the lowest percentage of subjects (2 subjects, 7%) reported AEs after receiving eluxadoline alone. All AEs were mild in severity and resolved by the end of study. There were no deaths, serious AEs (SAEs), or AEs leading to study drug discontinuation. The highest percentage of subjects overall (6 subjects, 20%) reported AEs classified as GI disorders. Gastrointestinal AEs were reported by 4 subjects (13%) after receiving eluxadoline in combination with cyclosporine, 2 subjects (7%) after receiving eluxadoline combined with probenecid, and 1 subject (3%) after receiving eluxadoline alone. Nausea was the most frequently reported GI AE (4 subjects overall, 13%), and was reported by 3 subjects (10.0%) after receiving eluxadoline combined with cyclosporine and 1 subject (3%) after receiving eluxadoline with probenecid. Nausea was not reported after administration of eluxadoline alone. Overall, clinical findings from 12-lead ECGs after dosing were similar to those at baseline and no individual 12-lead ECG abnormality was considered clinically significant or reported as an AE by the investigator. No individual clinical laboratory abnormality was considered clinically significant or reported as an AE by the investigator.

Discussion

Nonclinical in vitro and in vivo studies had established that eluxadoline was poorly absorbed after oral administration and eliminated primarily by hepatobiliary excretion with the absence of any significant metabolism. Other exploratory in vitro studies indicated that eluxadoline was a substrate of MRP2, OAT3, and OATP1B1 and an inhibitor of OATP1B1.

OATP1B1 is expressed at the sinusoidal membrane of the hepatocyte,[6,7] whereas OAT3 is expressed at the basolateral membrane of the renal proximal tubule cell,[6] and MRP2 is localized to the apical membranes of the hepatocyte (canalicular membrane), renal proximal tubule cell, and enterocyte.[6,12]

Cyclosporine has been established as an in vitro and in vivo inhibitor of OATP1B1 and OAT3.[6–10] Furthermore, cyclosporine has been shown to be a potent in vitro inhibitor of MRP2[13] as it binds to MRP2 with a Ki of 10–21 μM,[14] inhibits MRP2,[6] and at 10 μM demonstrates sustained inhibition of MRP2 (limited by the turnover rate of MRP2 [approximately 24–72 hours]). Additional support that cyclosporine is an in vivo inhibitor of MRP2 came from an in vivo DDI study of cyclosporine and mycophenolate mofetil, a prodrug of mycophenolic acid (MPA [a substrate of MRP2]).[15,16] Also, in vivo DDI studies coadministered with cyclosporine (using single doses of 100–300 mg) confirm that the cyclosporine dose of 600 mg used in our study provides adequate cyclosporine plasma concentration to reasonably evaluate any OATP1B1- and MRP2-mediated interactions with eluxadoline that may be present.[17–20]

Probenecid has also been identified as an inhibitor of OATP1B1 and OAT3.[6,10] As is the case for cyclosporine, probenecid, at a Ki of 44.6 μM, was identified not only as an inhibitor of MRP2,[21–23] but also as a substrate of MRP2,[24] as MRP2 mediates probenecid elimination via the bile.[25] Therefore, cyclosporine and probenecid were chosen as prototypical inhibitors to assess the effects of OATP1B1, OAT3, and MRP2 inhibition on the absorption and systemic disposition of eluxadoline.

Prior evidence of eluxadoline's poor oral bioavailability in humans had been suggested by: (a) poor in vitro GI permeability studies in Caco2 cell lines, and (b) its zwitterionic nature leading to a negatively charged molecule across the GI pH range.

From our study, human F_{oral} for eluxadoline was estimated to be 1.02%, primarily due to poor F_{GI} (2.3%), but also due to moderate pre-systemic ER_{hep} (55.8%). Estimated biliary clearance (832 mL/min) exceeded CL_{ren} (116 mL/min), indicating that hepatobiliary rather than renal excretion is the major elimination pathway for eluxadoline. Intrinsic biliary CL_{int} (9,370 mL/min) was high and exceeded hepatic blood flow, but was limited by plasma protein binding ($f_u = 19\%$) as was net CL_{TS} (459 mL/min).

Given the uncertainty in estimating the human CL_{TOT} value by allometric PK scaling, we performed a sensitivity analysis of the physiological clearances and absorption parameters relative to CL_{TOT}. Within a range of 500–1,200 mL/min for CL_{TOT}, the ADME/PK model parameter estimates are not only biologically plausible, but also reasonable (see Figure 3). Finally, as discussed above, the model-predicted AUC and Cl_{ren} values were very similar to their corresponding observed (geometric

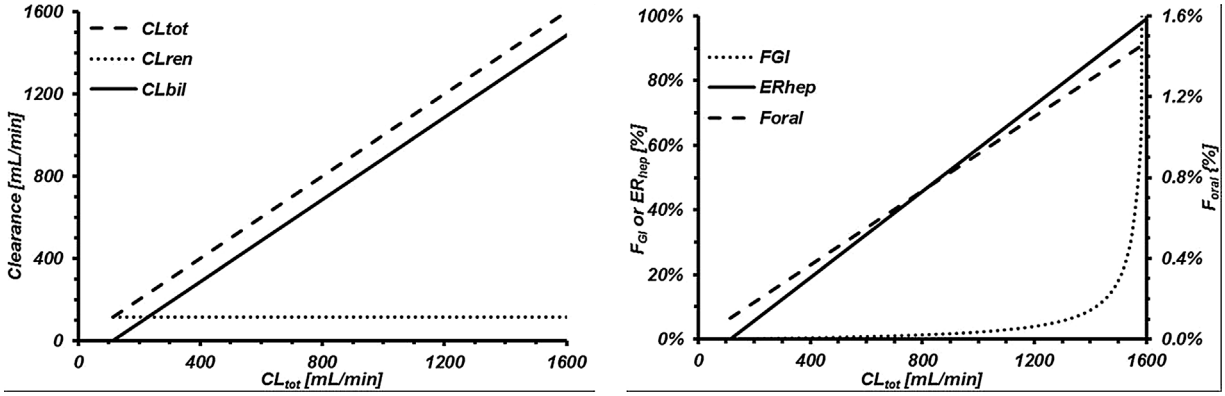


Figure 3. Sensitivity plots of PK parameters to changes in allometric CL_{TOT} estimate.

mean) values, confirming the validity of the semi-physiological model and parameter estimates.

Increased exposure to eluxadoline in the presence of cyclosporine and probenecid as seen in our study indicates increased oral bioavailability and/or reduced systemic clearance of eluxadoline whereas higher mean $t_{1/2}$ values for eluxadoline with cyclosporine and probenecid compared to eluxadoline alone suggests systemic clearance was reduced by both transporter inhibitors. It should be noted that in some subjects low concentrations of eluxadoline around the LLQ (0.100 ng/mL) were observed out to 72 hours resulting in inability to estimate the terminal rate constant. Additionally, some plasma concentration time profiles suggest evidence of enterohepatic recycling which may also have contributed to the lack of ability to estimate terminal rate constants. However, as noted earlier, 70% of eluxadoline and eluxadoline + probenecid and 63% of eluxadoline + cyclosporine concentration time profiles had estimable terminal elimination rates.

If early concentrations of eluxadoline were related to active efflux transport at the intestinal epithelial surface, inhibition of intestinal MRP2-mediated efflux by cyclosporine or probenecid would have been expected to result in increased early plasma concentrations. Cyclosporine had no effect on eluxadoline early plasma concentration at 0.25 and 0.5 hours after dosing when inhibition of MRP2 intestinal transporters would be expected to demonstrate an effect. Conversely, some increases in eluxadoline plasma concentrations were observed with probenecid at both 0.25 and 0.5 hours. However, variability in eluxadoline concentrations at early time points in the presence of probenecid was such that no definitive conclusion could be reached. Thus, any interactions of eluxadoline with MRP2 at the enterocytes play only a minimal, if any, role in the oral absorption of eluxadoline.

On the other hand, by 1.0 hour after dosing, it is likely that hepatic transporter inhibition predominates, and large increases in plasma concentrations of eluxadoline were observed when administered with cyclosporine, whereas no changes in plasma eluxadoline concentrations were observed when administered with probenecid (relative to plasma concentrations when eluxadoline was administered alone). In the presence of cyclosporine, the upper bound of the 90% confidence interval exceeded 5.0 for both C_{max} and AUCs (for both ANOVA approaches), indicating a strong DDI (Table 2). Based on the limited impact of any MRP2 inhibition at the intestine and kidney by cyclosporine (see below) and at the liver by probenecid, the magnitude of the DDI by cyclosporine strongly suggests inhibition of OATP1B1-mediated sinusoidal uptake by cyclosporine. In the absence of hepatic metabolism, inhibition of sinusoidal OATP1B1-mediated hepatic uptake by cyclosporine substantially reduces both hepatic first-pass extraction and systemic biliary clearance of eluxadoline.

As the estimated CL_{ren} for eluxadoline in the absence of any inhibitor exceeded glomerular filtration rate (GFR), one can conclude the presence of net renal tubular secretion, presumably reflecting transporter-mediated uptake into and efflux of eluxadoline out of proximal renal tubular epithelial cells. Probenecid, by inhibiting CL_{ren} from 116 to 62 mL/min reduces the fraction of the dose renally excreted unchanged (%Fe) from 0.12% to 0.08% despite the small (1.3-fold) observed increases in total exposures of eluxadoline. On the other hand, cyclosporine slightly decreases CL_{ren} from 116 to 97 mL/min but increases %Fe from 0.12% to 0.46%, as a result of the 6.1-fold increased systemic eluxadoline exposures (due to its potent inhibition of hepatobiliary excretion). Clinically, however, as renal excretion is only a minor elimination pathway for eluxadoline, the observed reductions in CL_{ren} are not expected to be clinically relevant.

Therefore, the strong DDI of cyclosporine and the mild DDI of probenecid with eluxadoline provide evidence that hepatobiliary excretion, eluxadoline's major elimination pathway, is affected predominantly by cyclosporine,

whereas renal excretion, its minor elimination pathway, is affected primarily by probenecid. This in turn suggests that hepatic OATP1B1 is likely the main transporter of interest in the absorption (ie, first-pass extraction) and systemic disposition of eluxadoline. The final parameters from the semiphysiological PK/ADME model indicate the following: biliary CL_{int} is reduced by 71% and 21% in the presence of cyclosporine and probenecid, respectively. Based on their known inhibitory potency for the transporters of interest, these reductions reflect strong inhibition of OATP1B1-mediated hepatic uptake of eluxadoline by cyclosporine and mild inhibition of MRP-2-mediated and/or contribution of other transporters to the canalicular efflux of eluxadoline by probenecid. These reductions in CL_{int} increase F_{oral} from 1.02% to 1.82% and 1.15%, while decreasing CL_{bil} from 836 to 296 mL/min and 750 mL/min in the presence of cyclosporine and probenecid, respectively. CL_{TS} is reduced by 24% and 52% in the presence of cyclosporine and probenecid, respectively. These reductions reflect mild inhibition of renal tubular apical/luminal MRP2-mediated efflux of eluxadoline by cyclosporine and the expected inhibition of OAT3-mediated basolateral uptake of eluxadoline by probenecid. However, as noted above, the observed changes in CL_{ren} are smaller than the reductions in CL_{TS} as the glomerular filtration of eluxadoline is not affected by cyclosporine and probenecid.

In conclusion, the results of this study provide evidence that eluxadoline has poor oral bioavailability (1.02%) in humans, primarily due to low GI permeability (F_{GI} of 2.3%), but also hepatic first-pass extraction (55.8%). Renal excretion of eluxadoline was found to be a minor pathway (12%) of overall elimination, with OAT3 being the main transporter involved in renal tubular secretion. Therefore, OAT3 inhibition due to probenecid leads to only a minor systemic DDI with eluxadoline. Hepatic/canalicular and renal apical/luminal MRP2-mediated efflux are only minimally affected by cyclosporine and probenecid, suggesting that MRP2 inhibition is not likely to lead to any major systemic DDI with eluxadoline. Most importantly, OATP1B1-mediated hepatic uptake with subsequent biliary excretion plays a key role in hepatic extraction of eluxadoline. Thus, OATP1B1 inhibition is likely to lead to a major DDI with eluxadoline, both by increasing its oral bioavailability (due to decreased hepatic first-pass extraction) and reducing its systemic (biliary) clearance.

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