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In Vitro Drug-Drug Interaction Evaluation of Epetraborole, a Novel Bacterial Leucyl-tRNA Synthetase Inhibitor

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Abstract

Background

Epetraborole (EBO) is a boron-containing oral inhibitor of bacterial leucyl-tRNA synthetase, an essential enzyme in protein synthesis; EBO demonstrates potent activity against nontuberculous mycobacteria. EBO is being developed for the treatment of *Mycobacterium avium* complex lung disease patients and will be used in combination with other drugs. Therefore, EBO and its major circulating metabolite M3 were evaluated in a comprehensive drug-drug interactions (DDI) risk assessment.

Methods

Stability of EBO was evaluated in human liver microsomes, hepatocytes and recombinant CYP enzymes. The inhibitory potential of EBO (0.03-100 µM) and M3 (1-1000 µM) on cytochrome P450 (CYP) activities was assessed using human hepatic microsomes. The CYP induction potential of EBO (25-200 µM), M3 was evaluated and compared to prototypical inducers in human hepatocytes (three donors) and mRNA. Fold increase in mRNA expression was utilized to investigate the CYP induction potential of EBO (0.3-100 µM) and M3 (1-250 µM). Stably transfected cell lines that expressed individual transporters were used to determine whether EBO or M3 were substrates or inhibitors for these proteins.

Results

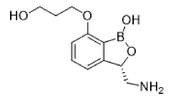
In vitro studies with microsomes, hepatocytes and recombinant cytochrome P-450 (CYP) enzymes indicated that EBO was a poor substrate for major CYP enzymes; drug interactions with epetraborole as victim are considered unlikely. Neither EBO nor its major metabolite M3 was a potent reversible or time-dependent inhibitor of major CYP enzymes. Half maximal inhibitory concentration (IC50) values for CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4 were >100 µM. EBO was not an inducer of CYP1A2 mRNA in human hepatocytes from three donors, while it was a weak inducer of CYP2B6 and CYP3A4. No induction of mRNA was observed in human hepatocytes at concentrations relevant to planned clinical doses. EBO is unlikely to be a substrate for P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, MATE1 and MATE2K. EBO was an in vitro substrate for OCT2, a transporter involved in active renal secretion. At clinically relevant concentrations, neither EBO nor M3 inhibited major human efflux or uptake transporters.

Conclusions

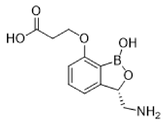
At clinically relevant concentrations of EBO and its major circulating metabolite M3, there is a low risk of victim or perpetrator DDI.

Introduction

The nontuberculous mycobacteria (NTM) are a phenotypically-diverse group of species and subspecies found throughout the environment. Lung disease is the most common manifestation of human NTM infection, and *Mycobacterium avium* complex (MAC) is the most frequent cause of NTM lung disease. Current standard of care (SOC) therapy for MAC lung disease requires administration of multiple drugs including clarithromycin and rifampin or rifabutin and ethambutol for 1-2 years. Objectives of the current studies were to investigate if EBO and its primary metabolite, M3 alter the pharmacokinetic properties of SOC drugs or vice versa and affect their efficacy and/or safety.



Chemical structure of EBO



Chemical structure of M3

METHODS & RESULTS

Table 1 Epetraborole Metabolic Stability

Test System	Species	[Test Article] (µM)	Incubation Conditions	Method of Analysis	Positive Control	Results
Liver microsomes	Rat, dog, NHP, human	EBO (1 or 10)	37°C for 0, 15, 30, 60, and 120 min	LC-MS/MS	Dextromethorphan	EBO was stable in the presence or absence of NADPH in all species.
Hepatocytes	Rat, dog, NHP, human	EBO (1 or 20)	37°C and 5% CO2 for 0, 30, 60, and 120 min	LC-MS/MS	Dextromethorphan	EBO was stable in all species after 4 h incubation
Hepatocytes	Human (3 donors)	^{14}C-EB0 (10 or 50)	37°C and 5% CO_2 for 0, 15, 30, 60, 120, and 240 min	HPLC with radiochemical detection and LC-MS	7-ethoxycoumarin	^{14}C-EBO accounted for >91% of total radioactivity in human hepatocytes (3 donors) after 120 minutes of incubation at both test article concentrations. Low levels of M3 was detected
HepaRG Cells	Human cell line	^{14}C-EB0 (5 or 500)	37 °C and 5% CO_2 for 4 and 24 hours	Liquid Scintillation counter and Radio-HPLC	7-ethoxycoumarin	There was no detectable metabolism of ^{14}C-EB0 ± NADPH and NAD+ cofactor regeneration systems
Aroclor 1254 and Phenobarbital-5,6-benzoflavone induced rat liver S9	Rat	^{14}C-EB0 (5 or 500)	37 °C for 180 min	Liquid Scintillation counter and Radio-HPLC	7-ethoxycoumarin	There was no detectable metabolism of ^{14}C-EB0 ± NADPH and NAD$^+$ cofactor regeneration systems
Recombinant CYP2E1 Enzymes	Human	^{14}C-EB0 (10 or 100)	37°C for 0, 15, 60, and 120 min	Liquid scintillation counter and Radio-HPLC	Chlorzoxazone	No M3 formation
Purified ADH	*Saccharomyces cerevisiae*	EB0 (1) and 4-MP (600)	RT	Spectrophotometer (340 nm)	-	EBO increased NADH formation in a time dependent manner NADH formation was completely inhibited by 4-MP, suggesting possible EBO oxidation by ADH resulting in the formation of an aldehyde intermediate in vitro
ADH inhibition in NHP	NHP	EBO 35 mg/kg/day (IV) 4-MP 35 mg/kg/day (PO)	-	LC-MS/MS	-	In the presence of 4-MP, mean systemic exposure was increased for EBO ($AUC_{0\text{-}t}$ 72.6 vs. 164.5 µg.h/mL) and decreased for M3 ($AUC_{0\text{-}t}$ 55.2 vs. 10.2 µg.h/mL)

NHP=nonhuman primate; EBO=epetraborole; AUC=area under the curve; HPLC=high performance chromatography; RT=room temperature; ^{14}C=carbon 14; NADPH=nicotinamide adenine dinucleotide phosphate; NAD=nicotinamide adenine dinucleotide; 4-MP=4-methyl pyrazole, LC-MS/MS=liquid chromatography with tandem; mass spectrometry; IV=intravenous; PO=oral, ADH=alcohol dehydrogenase

Table 2 Effect of Epetraborole on Cytochrome P450 Isoenzymes

Test System	Species	[Test Article] (µM)	Incubation Conditions	Method of Analysis	Positive Control	Results
Hepatic microsomes (Inhibition)	Human	0.03-100	37°C for 0.75-30 min	LC/MS/MS	Fluvoxamine (CYP1A2), thiotepa (CYP2B6), montelukast (CYP2C8), sulfaphenazole (CYP2C9), omeprazole (CYP2C19), quinidine (CYP2D6), diethyldithiocarbamate (CYP2E1), and ketoconazole (CYP3A)	Mild (30%) inhibition of CYP1A2 at 100 µM ± NADPH No inhibition of CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 ± NADPH
Hepatocytes (Induction)	Human (n=3 donors)	0.3-100	37°C and 5% CO_2 for 2 days	RT-PCR	Omeprazole phenobarbital Rifampin	Not an inducer of CYP1A2 Weak inducer of CYP2B6 and CYP3A4 at 100 µM (2.18- & 2.93-fold increase in mRNA expression, respectively)

No IC_{50} values could be calculated. LC-MS/MS= liquid chromatography with tandem mass spectrometry; RT-PCR=reverse transcription- polymerase chain reaction; mRNA=messenger ribonucleic acid; NADPH=nicotinamide adenine dinucleotide phosphate; IC_{50}=concentration associated with 50% inhibition

Table 3 Effect of M3 on Cytochrome P450 Isoenzymes

Test System	Species	[Test Article] (µM)	Incubation Conditions	Method of Analysis	Positive Control	Results
Hepatic microsomes (Inhibition)	Human	1-1000	37°C for 5 or 10 min	LC/MS/MS	Phenacetin (CYP1A2), rosiglitazone (CYP2C8), diclofenac (CYP2C9), bufuralol (CYP2D6) atorvastatin (CYP3A4) midazolam (CYP3A4) nifedipine (CYP3A4)	No inhibition of CYP1A2, CYP2C8, CYP2C9, CYP2D6, and CYP3A4
Hepatocytes (Induction)	Human (n=3 donors)	1-250	37°C and 5% CO_2 for 2 days	RT-PCR	Omeprazole phenobarbital Rifampin	Not an inducer of CYP1A2 & CYP2B6 Weak inducer of CYP3A4 at 100-250 µM (2.19- & 2.13-fold increase in mRNA expression, respectively) with EC_{50} of 32.44 µM

No IC_{50} values could be calculated; LC-MS/MS= liquid chromatography with tandem mass spectrometry; RT-PCR=reverse transcription- polymerase chain reaction; mRNA= messenger ribonucleic acid; NADPH=nicotinamide adenine dinucleotide phosphate; IC_{50}=concentration associated with 50% inhibition; EC_{50}= half maxima effective concentration

Table 4 Summary of In Vitro Evaluation of Transporter-Mediated Drug Interaction Potential of Epetraborole and M3 in Human Cells

Test System	Transporter	Epetraborole as an Inhibitor, IC_{50} (µM)	Epetraborole as a Substrate	M3 as an Inhibitor, IC_{50} (µM)	M3 as a Substrate
HEK 293, HEK MSRII, MDCK, MDCKII-BCRP, MDCK-MDR1, and Caco-2-cell lines transfected with the uptake transporters	OATP1B1	25% inhibition at 500 µM	No	>250	No
	OATP1B3	21% inhibition at 500 µM	No	>250	No
	OCT1	59% inhibition at 1000 µM	ND	>250	ND
	OCT2	20% inhibition at 1000 µM	Yes	>250	No
	OAT1	>1000	No	>250	No
	OAT3	>1000	No	>1000	No
	MATE1	>100	No	>250	No
	MATE2K	>100	No	>250	No
	P-gp	5651	No		No
	BCRP	27% inhibition at 1000 µM	No	>500	No

BCRP=breast cancer resistance protein; IC_{50}=concentration associated with 50% inhibition; ND=not determined; OAT=organic anion transporter; OATP= organic anion transporting polypeptide; OCT=organic cation transporter; P-gp=P-glycoprotein

CONCLUSIONS

- Cytochrome P-450 (CYP) enzyme system is not involved in EBO metabolism
  - At clinically relevant concentrations, EBO and M3 have minimal to no inhibitory or induction effect on the major CYP enzymes
  - In human volunteers receiving EBO at 500 mg QD PO achieved plasma
  - EBO and M3 C_{max} values of 12 µM and 20 µM, respectively (data not shown)
  - EBO is a weak inhibitor of CYP1A2 and a weak inducer of CYP2B6 & CYP3A4 at 100 µM which is ~8x the EBO plasma C_{max}
  - M3 is a weak inducer of CYP3A4 at 100 µM which is ~5x the M3 plasma C_{max}
- ADH is the major enzyme involved in the metabolism of EBO
- EBO and M3 showed either no inhibition or weak inhibition of transporters at concentrations that exceed the clinically relevant exposure
- Neither EBO nor M3 are substrates for the major human uptake and efflux transporters, with the exception of OCT2, a renal uptake transporter
  - Renal clearance of epetraborole is approximately 85 mL/min (data not shown), which is near or slightly less than glomerular filtration rate in humans and indicates that active renal secretion is not a major elimination route. Therefore, OCT2 mediated clinical DDI are unlikely
- Overall, at clinically relevant concentrations of EBO and its major circulating metabolite M3, there is very low risk of DDI

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