In vitro Drug Metabolism and Pharmacokinetic Evaluation of Ent-Verticilide

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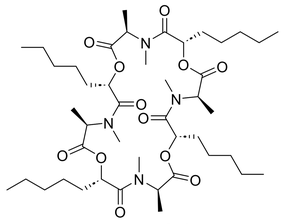
Introduction

BACKGROUND:

- Verticilide is a natural product of the fungus Verticillium FKI-1033.
- Verticilide is being evaluated as a potential therapeutic for the treatment of cardiac arrhythmias and/or neurodegenerative and seizure disorders associated with hyperactive ryanodine receptor type 2 (RyR2) calcium release channels.



***ent*-Verticilide**

- While the natural enantiomeric form of verticilide (*nat*-verticilide) demonstrates no activity against RyR2, the synthetically derived unnatural verticilide enantiomer (*ent*-verticilide) is a selective and potent inhibitor of cardiac RyR2 and exhibits antiarrhythmic activity in mice.
- Knowledge of the drug metabolism and pharmacokinetic (DMPK) properties of *ent*-Verticilide will be required to understand its therapeutic potential.

PROJECT GOAL: To evaluate the DMPK properties of *ent*-verticilide *in vitro*

OBJECTIVES:

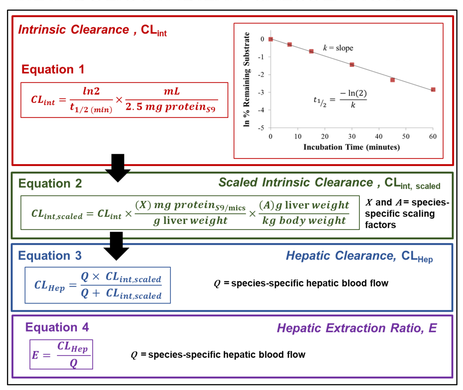
- Evaluation of metabolic stability in hepatic tissue of humans and preclinical species
- Metabolite profiling in humans and preclinical species
- Identification of enzymes responsible for human clearance

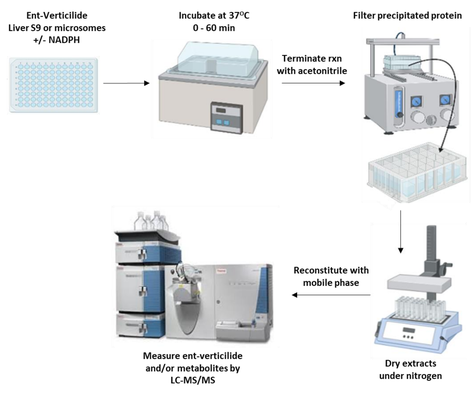
Methods

EXPERIMENTAL DESIGN:

- **Matrix:** Human, Mouse, Rat and Dog hepatic S9 or microsomes
- **Substrates:** *ent*-verticilide or verapamil (positive control)
- **CYP Inhibitors:** napthoflavone, 2-Phenyl-2-(1-piperidinyl)propane, montelukast, sulfaphenazole, N-3-benzylnirvanol, quinidine, or ketoconazole
- **Cofactor:** NADPH

METABOLIC STABILITY CALCULATIONS:

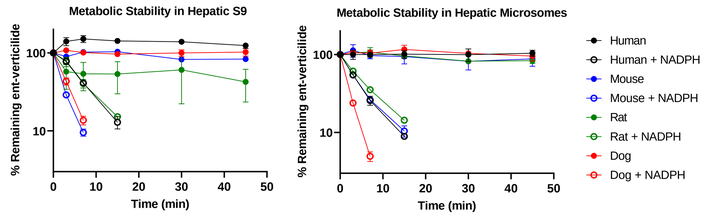




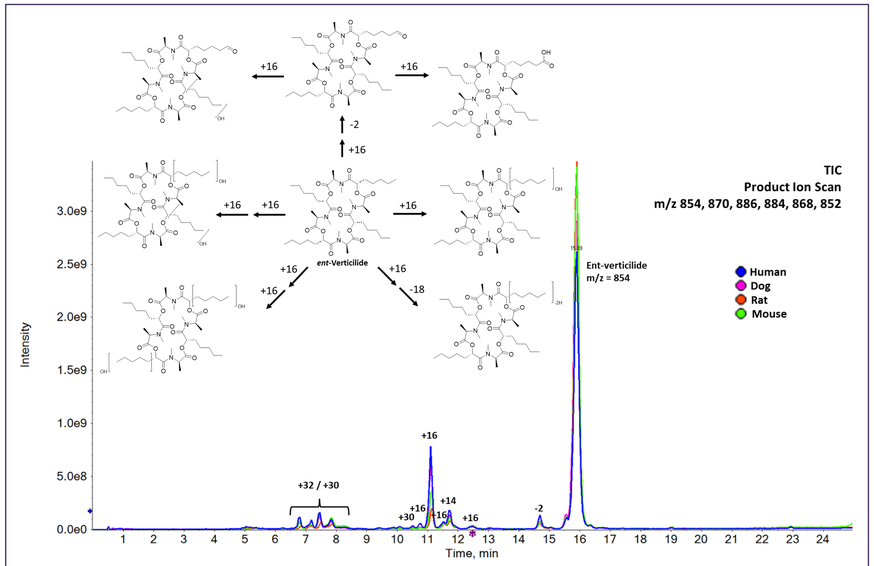
References: (1) Monma, S. et al. *Org Lett* 2006, 8 (24), 5601-4. **(2)** Batiste, S. M. et al. *Proc Natl Acad Sci* U S A 2019, 116 (11), 4810-4815. **(3)** Shiomi, K. et al. *J Antibiot* (Tokyo) 2010, 63 (2), 77-82.

Results

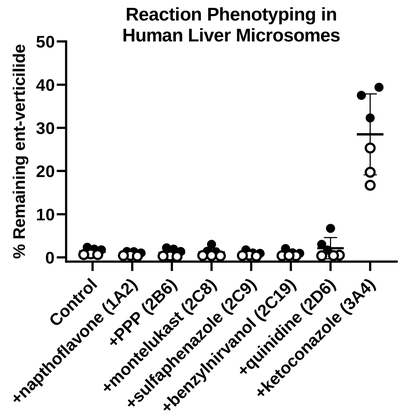
Species	Intrinsic Clearance (mL/min/kg)	Predicted Hepatic Clearance (mL/min/kg)	Predicted Hepatic Extraction Ratio
Mouse	1404 (913)	85 (82)	0.94 (0.91)
Rat	248 (623)	45 (50)	0.82 (0.91)
Dog	442 (1506)	29 (30)	0.94 (0.97)
Human	170 (255)	19 (19)	0.90 (0.90)



Metabolic Stability of *ent*-Verticilide in Mouse, Rat, Dog, and Human Liver Fractions. *ent*-Verticilide (1 μM) was incubated with hepatic S9 (2.5 mg/mL) or hepatic microsomes (0.5 mg/mL) at 37 ^{O}C in a potassium phosphate buffered reaction (100 mM, pH 7.4) in the presence or absence of the CYP450 cofactor NADPH (2 mM). Data represent means of a single experiment performed in triplicate. **Table:** S9 vales appear on the left and microsomal values appear in parentheses on the right. Because *ent*-Verticilide was stable in the absence of NADPH, only NADPH-dependent clearance values are reported.



Proposed Metabolites of *ent*-Verticilide Generated in Mouse, Rat, Dog, and Human Liver Microsomes. *ent*-Verticilide (10 μM) was incubated with hepatic microsomes (1 mg/mL) for 60 min at 37 ^{O}C in a potassium phosphate buffered reaction (100 mM, pH 7.4) in the presence of the CYP450 cofactor NADPH (2 mM).



Identification of Enzymes Contributing to Clearance of *ent*-Verticilide in Human Liver Microsomes. *ent*-Verticilide (1 μM) was incubated with hepatic microsomes (0.5 mg/mL) at 37 ^{O}C in a potassium phosphate buffered reaction (100 mM, pH 7.4) in the presence of the CYP450 cofactor NADPH (2 mM) with or without specific inhibitors for the major CYP450 enzymes. Data represent means ± SD of two experiments performed in triplicate.

Conclusions

Conclusions:

- *ent*-Verticilide was extensively metabolized by NADPH-dependent enzymes in mouse, rat, dog, and human liver S9 and microsomes and was stable in the absence of NADPH.
- NADPH-dependent substrate depletion suggests that *ent*-Verticilide is metabolized by cytochrome P450 enzymes.
- Reaction phenotyping in human liver microsomes suggests that CYP3A4 is primarily responsible for the metabolism of *ent*-verticilide.
- The proposed primary metabolites of *ent*-Verticilide include hydroxylation and oxidation of the alkyl chains, and formation of these metabolites was similar across species.