Understanding *Mitragyna speciosa* alkaloid metabolism and pharmacology in rat brain using imaging mass spectrometry

Poster #: WP 266

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OVERVIEW

- **Purpose:** To better understand corynantheidine metabolism, biodistribution, and contribution to the overall properties of kratom.
- **Approach:** LC-MS/MS was used to identify corynantheidine metabolites. Imaging mass spectrometry was used to map the distribution of corynantheidine and its metabolites.
- **Results:** Corynantheidine, corynantheidine-2H, and corynantheidine-4H were detected in rat brain. The first two molecules were localized to the corpus callosum while the third was localized to the neocortex.
- **Significance:** Corynantheidine metabolism was mapped in rat brain using imaging mass spectrometry to better understand alkaloid pharmacology.

INTRODUCTION

Mitragyna speciosa, more commonly known as kratom, has emerged as a self-prescribed alternative to opioid use for the treatment of chronic pain and addiction.[2] Due to potential detrimental health effects of its components, the pharmacological properties of each alkaloid component of kratom must be more fully characterized.[3]

Corynantheidine, a minor alkaloid of kratom, has been shown to bind to μ-opioid receptors, yet little is known about its metabolism, biodistribution, and contribution to the overall properties of kratom.[4] Here, we have used liquid chromatography-tandem mass spectrometry (LC-MS/MS) and imaging mass spectrometry (IMS) to identify and map the distribution of alkaloid metabolites in the brain to better understand the neurometabolism of these compounds.

a) b)

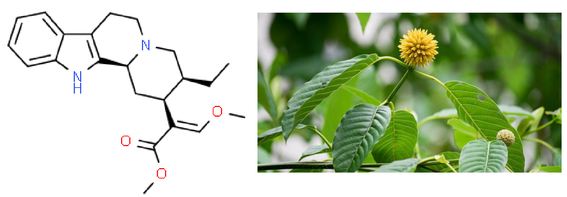

Figure 1. a) Chemical structure of corynantheidine. b) A kratom plant.

METHODS

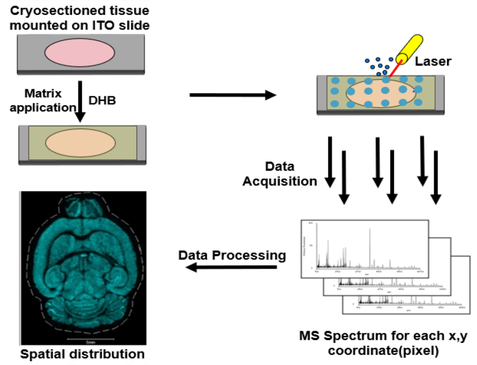

Figure 2. Imaging mass spectrometry workflow.

- **Tissue sample preparation:** One control male *Sprague Dawley* rat and one dosed with 10 mg/kg corynantheidine intravenously were sacrificed 30 minutes post-dose. The tissue was sectioned using a Leica CM3050S Cryostat.
- **Matrix application:** 2-4 mg of DHB matrix was applied to the slide using a custom-built sublimation apparatus.
- **IMS:** IMS was performed on a 7T solariX FT-ICR MS (Bruker Daltonics). CASI imaging was performed by setting the Q1 isolation window to *m/z* 369 ±2.5 Da, ±10 Da or ±25 Da.
- **Data calibration:**. The mass spectra were externally calibrated using a corynantheidine standard as the reference mass (Figure 3b).

RESULTS

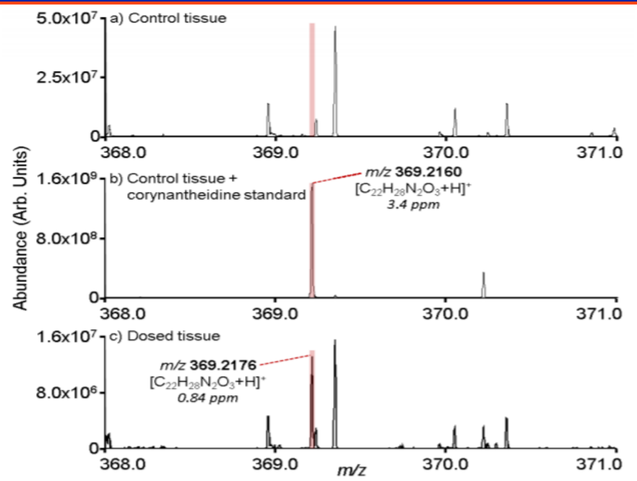

Figure 3. Mass spectra of a) control rat brain tissue, b) control rat brain tissue with addition of hand-spotted croynantheidine standard, and c) dosed rat brain tissue.[1]

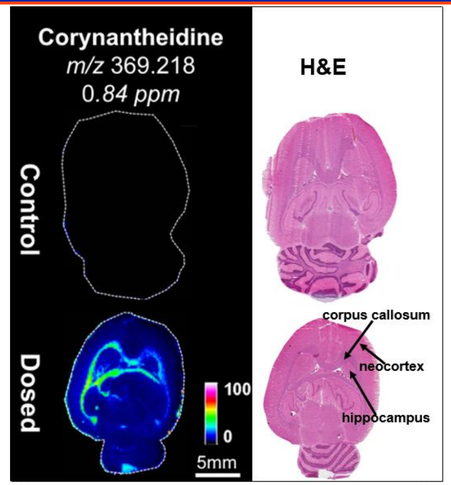

Figure 4. Spatial distribution of corynantheidiene in rat brain (200 *μm* spatial resolution) shown next to the H&E stained tissue images.[1]

CASI Window	*m/z* 351-356	*m/z* 365-370	*m/z* 371-376	*m/z* 381-386	*m/z* 400-405
Metabolites	Hydrolysis/demethylation (*m/z* 355.202)	Corynantheidine-2H (*m/z* 367.202)	Hydrolysis/demethylation+O (*m/z* 371.197)	Corynantheidine+O (*m/z* 385.212)	Corynantheidine+2O (*m/z* 401.207)
	Hydrolysis/demethylation -2H (*m/z* 353.186)	Hydrolysis/demethylation+O-4H (*m/z* 367.165)		Corynantheidine+O-2H (*m/z* 383.197)	
	Hydrolysis/demethylation-4H (*m/z* 351.170)	Corynantheidine-4H (*m/z* 365.186)		Corynantheidine+O-4H (*m/z* 381.181)	

Table 1. Corynantheidine metabolites identified by LC-MS/MS to be targeted by CASI imaging mass spectrometry.

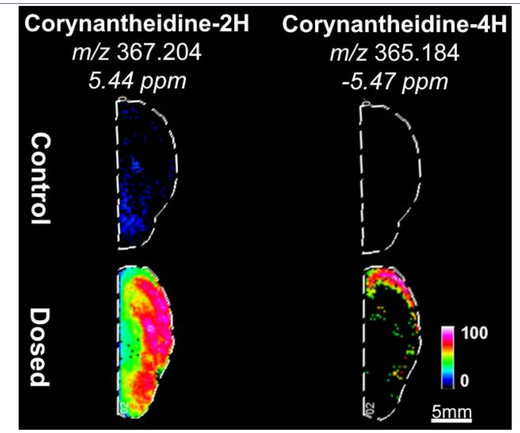

Figure 5. Spatial distribution of Corynanthidiene-2H and Corynanthidiene-4H metabolites in rat brain (250 *μm* spatial resolution).

CONCLUSIONS

- Corynantheidine and 2 of its metabolites were successfully detected in ratbrain tissue, demonstrating that corynantheidine readily crosses the blood-brain barrier.
- Corynantheidine and corynantheidine-2H are localized to the corpus callosum and parts of the hippocampus in the brain indicating possible interactions with μ- and δ- opioid receptors, adrenergic, and serotonin receptors.
- Future experiments will target other metabolites with higher spatial resolution, investigate how dose time effects the distribution, and examine the neurometabolism of other kratom alkaloids.

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