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Identifying the Human Blood Esterases Responsible for Metabolism of Etripamil, a Fast-Acting Calcium Channel Blocker Developed to Treat Paroxysmal Supraventricular Tachycardia

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Introduction

- Paroxysmal supraventricular tachycardia (PSVT) is an abnormal heart rhythm characterized by randomly occurring episodes of a rapid heart rate (>100 beats per minute) and can often result in significant symptoms including palpitations, chest discomfort, dyspnea, light headedness, syncope, and anxiety.
- PSVT results in 50,000 emergency department visits per year and is associated with significant healthcare resource utilization, including emergency department visits, and healthcare costs.[1]
- Acute episodes are managed through intravenous treatments like adenosine, non-dihydropyridine (NDHP) calcium channel blockers (CCBs), and β-blockers, which are administered in a healthcare setting.[2]
- Etripamil is a novel NDHP CCB formulated as a fast-acting intranasal spray for PSVT treatment that can be self-administered by patients without medical supervision.[3]
- Intranasal drug delivery yields a fast onset of action via rapid absorption into the bloodstream, which is clinically advantageous.
- In a phase 1 clinical trial, a 70 mg dose of intranasal etripamil (C_{max}) was rapidly absorbed through the nasal mucosa in ≤7 minutes (t_{max}).[4]
- Based on its chemical structure, etripamil is sensitive to metabolism by blood esterases,[3] which include butyrylcholinesterase (BChE), paraoxonase (PON), acetylcholinesterase (AChE), and albumin.
- Understanding the involvement of specific esterases that metabolize etripamil is needed to determine the role of esterases on the overall pharmacological effects of the drug.

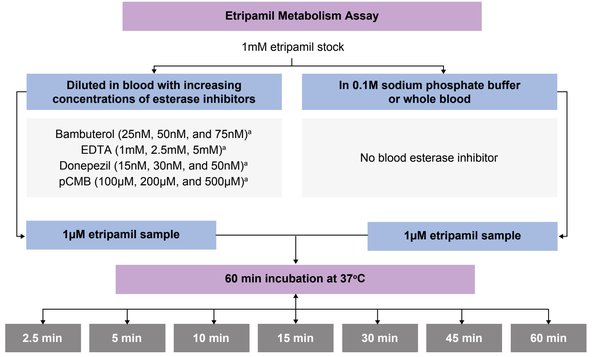
Objectives

- To understand how etripamil is metabolized in the human body and to identify the esterases responsible for etripamil metabolism.

Methods

- For the etripamil metabolism assay, a whole blood sample was obtained from a single human donor with consent and collected in 5-mL vacutainer tubes coated with sodium heparin.
- Blood was transported via cold chain logistics and received at a temperature of 4.8°C at the test laboratory.
- Etripamil-d3 HCl was used as the internal standard and was obtained from AptoChem. The compound was dissolved in dimethyl sulfoxide (DMSO) to prepare a 10mM stock solution, which was diluted to a final concentration of 1μM in 100% acetonitrile.
- The etripamil HCl salt was incubated in blood in the presence of specific esterase inhibitors, such as bambuterol for BChE, ethylenediaminetetraacetic acid (EDTA) for PON, donepezil for AChE, and p -chloromercuribenzoate (pCMB) for esterase D, to determine the esterase involved in etripamil metabolism (**Figure 1**).
- Liquid chromatography with mass spectrometry analysis was performed on an Ultra Performance Liquid Chromatography system (Waters) equipped with an AB Sciex QTRAP 6500 mass spectrometer, with the Analyst 1.6 software package (Applied Biosystems).
- A 2.1×50 mm, 2.5-μm column (Waters BEH C8) was used, with the flow rate maintained at 0.7 mL/min. The mobile phases were composed of water with 0.1% formic acid and acetonitrile with 0.1% formic acid.
- Etripamil concentration was calibrated using linear regression, and linearity was determined from 3nM to 3000nM (r^2=0.9991).
- Six iterations at three concentration levels were assessed on the same incubation day to analyze the accuracy and precision of etripamil concentration.

Figure 1. Etripamil Metabolism Assay



[a]A 10mM stock solution of bambuterol, EDTA, and donepezil, respectively, was prepared in 100% DMSO. The stock concentration of pCMB was 5mM prepared in double-distilled water. [b]Internal standard.
DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; min, minute; pCMB, p-chloromercuribenzoate.

Results

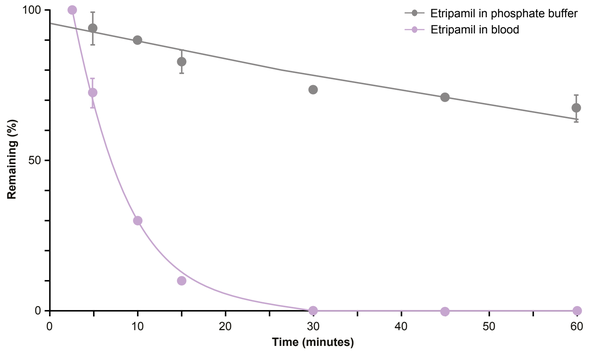
Etripamil Metabolism in Human Blood and Phosphate Buffer

- The concentration of etripamil decreased rapidly in human blood given that 10.4% of etripamil remained after 15 minutes of incubation and 0.45% remained after 1 hour (**Table 1** and **Figure 2**).
- In contrast, etripamil was not effectively metabolized in phosphate buffer. After 15 and 60 minutes of incubation, 83.6% and 68.0% of etripamil remained, respectively.

Table 1. Etripamil Metabolism in Phosphate Buffer Versus Blood

Incubation Time (min)	Etripamil in Phosphate Buffer		Etripamil in Blood	
	Concentration (nmol/L)	Remaining (%)	Concentration (nmol/L)	Remaining (%)
2.5	897	100.00	926	100.00
5	849	94.65	676	73.04
10	816	90.91	276	29.82
15	750	83.61	96	10.35
30	662	73.80	9	1.00
45	646	72.02	5	0.50
60	610	68.00	4	0.45

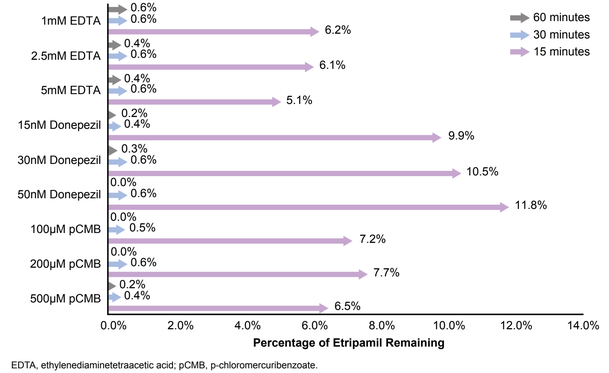
Figure 2. Etripamil Metabolism in Human Blood and Phosphate Buffer



Etripamil Metabolism With EDTA, Donepezil, and pCMB

- Etripamil metabolism in blood was not affected by incubation with different concentrations of blood esterase inhibitors EDTA, donepezil, and pCMB.
- After 15 minutes of incubation with blood and the highest concentration of respective esterase inhibitors, 5.1% with 5mM EDTA, 11.8% with 50nM donepezil, and 6.5% with 500μM pCMB remained (**Figure 3**).

Figure 3. Etripamil Metabolism in Blood With Different Concentrations of Blood Esterase Inhibitors



EDTA, ethylenediaminetetraacetic acid; pCMB, p-chloromercuribenzoate.

- Incubation of etripamil with the blood esterase inhibitor bambuterol led to a concentration-dependent metabolism of etripamil (**Figure 4**).
- After 15 minutes of incubation, 31.8% (50nM bambuterol) and 43.2% (75nM bambuterol) of etripamil remained; after 60 minutes of incubation, 4.8% and 17.4% remained, respectively (**Figure 4** and **Table 2**).

Figure 4. Etripamil Metabolism in Blood With Bambuterol

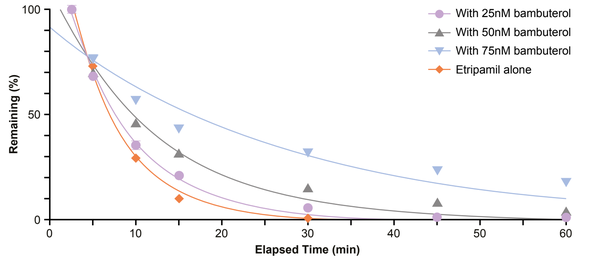


Table 2. Etripamil Metabolism in Blood Containing Bambuterol

Incubation Time (min)	25nM Bambuterol		50nM Bambuterol		75nM Bambuterol	
	Concentration (nmol/L)	Remaining (%)	Concentration (nmol/L)	Remaining (%)	Concentration (nmol/L)	Remaining (%)
2.5	863	100.00	1028	100.00	916	100.00
5	589	68.29	721	70.17	701	76.57
10	306	35.42	473	46.03	522	56.96
15	184	21.28	327	31.82	395	43.15
30	47	5.41	157	15.28	290	31.62
45	13	1.47	88	8.53	215	23.43
60	6	0.71	49	4.77	160	17.42

Conclusions

- The study findings suggest that the blood esterase BChE, targeted by the inhibitor bambuterol, is primarily responsible for the metabolism of etripamil in the human body.
- Further studies to characterize the metabolism of etripamil via BChE are in progress.

References

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Disclosures

DW and **DB** are employees of Milestone Pharmaceuticals. **J-PM** is an employee of Recherche Continuum Research.

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