Areca Nut Toxicokinetics: CES1 Catalysis and Alcohol Inhibition of Arecoline Disposition

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Background/Objectives: The highly toxic areca nut is the 4th most abused psychoactive substance in the world and is a known carcinogen, causing oral and hepatic cancers. The elimination of arecoline, the primary toxin in the areca nut, in humans is metabolically a mystery. Furthermore, epidemiological data demonstrates that areca nut chewers who consume alcohol have increased toxicities, yet the mechanism remains undescribed. Henceforth, our objectives in this project were to identify the precise carboxylesterase that catalyzes arecoline hydrolysis (de-esterification), and quantify alcohol's effect on arecoline hydrolysis.

Experimental Methods: Arecoline (50 μM) was incubated at 37°C (pH 7.4) in the presence of carboxylesterase 1 (CES1) or CES2, two major esterases expressed in human liver. Incubations were conducted under increasing reaction times (10 to 40min) or increasing enzyme concentration (0.1 to 0.4mg/ml). Depletion of arecoline and formation of metabolite (arecaidine) was monitored by a validated ion exchange high-performance liquid chromatography (HPLC) assay. Furthermore, inhibition of arecoline metabolism was quantified in the presence of varying concentrations of alcohol (10 – 340mM), and IC_{50} was determined via non-linear regression utilizing GraphPad Prism software.

Results: Arecoline was preferentially de-esterified by CES1, with no contribution by CES2. CES1-mediated metabolism of arecoline was significantly correlated with reaction time ($p<0.01$) and enzyme concentration ($p<0.05$). In the presence of alcohol, CES1-mediated formation of arecaidine was reduced, with a mean (±SD) IC_{50} of 36.3mM (±5.74), which is equivalent to 0.17g/dL (±0.024).

Conclusion: For the first time, we show CES1 is the chief carboxylesterase that catalyzes de-esterification of arecoline to arecaidine, a less toxic metabolite. We are also the first to demonstrate arecoline hydrolysis is inhibited by alcohol concentrations that are physiologically relevant in drinkers. The arecoline-alcohol interaction may explain toxicities observed in areca nut chewers that also ingest alcohol, a premise to be studied in future cell culture and animal models.

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