In vitro – In silico Investigation of Therapeutic Medicines that Inhibit Arecoline Hydrolysis

Uyen Pham, Mahendran Jayakumar, Shubham Srivastava, and Alan L. Myers

Department of Diagnostic & Biomedical Sciences, School of Dentistry, The University of Texas Health Science Center at Houston (Houston, TX)

Background/Objectives: Arecoline, a naturally occurring alkaloid from the areca (betel) nut, exerts multiple psychoactive effects and damages oral tissues. Given its high abuse potential and toxicodynamic profile, drug interactions that result in increased arecoline exposure could have drastic health consequences in areca nut (AN) consumers. Despite widespread consumption since antiquity, inhibitors of arecoline metabolism are still unidentified. We hypothesize that medications from various pharmacological classes significantly slow down the liver's ability to eliminate arecoline.

Experimental Methods: Our hypothesis was initially tested by elucidating the kinetics of the ubiquitous human carboxylesterase-1 (hCES1) substrate p-nitrophenyl acetate (pNPA) in the absence or presence of potential pharmacological inhibitors (e.g., statins, thyroid hormones, hormonal contraceptives, NSAIDs, muscle relaxants) using spectrophotometric assay. Next, drugs exhibiting significant inhibition (>60%) of pNPA hydrolysis were tested for inhibition of hCES1-mediated arecoline metabolism. A range of inhibitor concentrations were utilized to acquire their IC50. Inhibitors were further subjected to molecular modeling with the hCES1 protein using GLIDE module of Maestro v12.6 software.

Results: Amongst the tested therapeutics that significantly inhibited pNPA hydrolysis were lovastatin (76.2%), simvastatin (90.1%), L-thyroxine (90.2%), liothyronine (61%), celecoxib (60.5%), mefenamic acid (73.4%), progesterone (67.4%), norethindrone (87.2%), and ethinyl estradiol (66.5%). Their IC50 of arecoline hydrolysis were 1.80, 0.589, 0.0578, 10.1, 22.2, 8.77, 21.8, 2.65, and 5.74 µM, respectively. Molecular docking found that simvastatin, lovastatin, norethindrone, and L-thyroxine exhibited the greatest binding affinity. Furthermore, binding kinetics were significantly correlated with inhibitor effects, which were greatly dependent on hydrophobic interactions within hCES1 active site.

Conclusion: Remarkably, therapeutic dosage of L-thyroxine (T4) renders a plasma concentration that exceeds its IC50 reported herein, indicating the possibility of clinically significant interaction with arecoline in AN consumers. In addition, the intriguing finding endogenous thyroid hormones, primarily T4, potently inhibit hCES1 metabolism opens new questions about other biological interplay with CES1 functionality in humans.

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