In vitro and In vivo Inhibition of Nicotine Metabolism by \textit{trans-}2-Nitrocinnamaldehyde

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Background: Pharmacogenomic studies show variation in nicotine metabolism influences smoking behaviors; slow metabolizers exhibit a decrease in smoking frequency. Thus, the design of inhibitors of nicotine metabolism is one approach for the discovery of new cessation therapies. Previous in vitro evidence demonstrate that \textit{trans-}2-methoxycinnamaldehyde and \textit{trans-}cinnamaldehyde are time-dependent inhibitors of human CYP2A6, the enzyme responsible for nicotine clearance. In vitro-to-in vivo extrapolation predicts a > 4-fold change in nicotine AUC (area under the curve). Here we evaluated the structural analog, \textit{trans-}2-nitrocinnamaldehyde (NCA) in vitro, using human and mouse liver microsomes, and in vivo in C57GL/6J mice.

Hypothesis: The addition of the nitro group will stabilize a purported terminal formyl radical and lead to greater in vitro and in vivo inhibition potency of nicotine metabolism.

Methods: In vitro time-dependent inhibition studies were conducted by monitoring the remaining activity of nicotine metabolism after incubating NCA (0-120 \textmu M) with human (2 mg/mL) and mouse liver microsomes (2 mg/mL) for 0 to 90 minutes. An aliquot was transferred to the secondary incubation system containing NADPH (1mM), nicotine (50 \textmu M), and corresponding liver cytosol (1 mg/mL) at specific time points. After incubation for 15 minutes at 37 \textdegree C, each secondary incubation was quenched with ice-cold acetonitrile and the internal standard (D3-cotinine) was added. Cotinine was quantified using a Sciex 3500 LC-MS/MS. For in vivo studies, using adult male C57BL/6J mice, two modes of administration were selected. For oral administration, NCA was dissolved in the drinking water at the concentration of 0.088 mg/mL, to which the mice had access for three days prior to intraperitoneal injection of nicotine (1 mg/kg). To compare potency with methoxsalen (a known CYP2A6 inhibitor) NCA (57.1 mg/kg) was also administered via intraperitoneal injection. Blood was collected at specific time points (5 to 120 minutes). Samples were centrifuged to isolate plasma, D3-cotinine (internal standard) and acetonitrile were added, and then centrifuged. Supernatants were analyzed on LC-MS/MS to quantify nicotine and cotinine using standard curves generated in mouse plasma. Blood concentrations from four to five mice were measured for each time point and AUC values were estimated using Phoenix WinNonlin.

Result: The in-vitro study using mouse liver microsomes showed that NCA time-dependently inhibited the formation of cotinine from nicotine with a \( k_{\text{inact}} \) of 0.060 min\(^{-1}\) (± 0.024) and a \( K_I \) of 6.0 \textmu M (± 2.0). Oral administration of NCA in mice resulted in a nicotine plasma AUC of 355.9 ng\textsuperscript{\textast}min/mL (geometric mean; 95% CI: 151.5 – 836.6), which is estimated to be 2.5 times higher than the control nicotine plasma AUC of 143.3 ng\textsuperscript{\textast}min/mL (geometric mean; 95% CI: 112.2 – 183.0). Intraperitoneal injection of NCA and methoxsalen resulted in nicotine plasma AUC of 280.7 ng\textsuperscript{\textast}min/mL (geometric mean; 95% CI: 170.7 – 474.7; AUC-fold change = 1.96) and 957.6 ng\textsuperscript{\textast}min/mL (geometric mean; 95% CI: 532.4 – 1665.8; AUC-fold change = 6.68), respectively.

Conclusion: NCA is a more potent in vitro time-dependent inhibitor of nicotine metabolism compared to \textit{trans-}2-methoxycinnamaldehyde and \textit{trans-}cinnamaldehyde. Greater inhibition was observed with oral administration compared to intraperitoneal injection; this is evidence that NCA is reasonably stable in water to allow for oral dosing.