Identification of 5-hydroxycotinine in the Plasma of Nicotine-treated Mice-Implications for Cotinine Metabolism and Disposition in vivo

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Introduction: Nicotine, the addictive ingredient of tobacco, is metabolized in the liver to cotinine - the stable biomarker of nicotine use. Cotinine is further oxidized to 3-hydroxycotinine (as detected in the plasma) for renal excretion. In mice, these reactions are catalyzed by the microsomal enzyme CYP2A5 which closely resembles human CYP2A6.

Aim: Is 3-hydroxycotinine the only major oxidation product of cotinine?

Methods: Mice were injected with nicotine (1 mg/kg s.c.) or exposed to e-cigarettes containing 2.4% nicotine for 4 h. Blood was collected 15 min post-injection, or 5-6 min after the end of e-cigarette exposure. Plasma nicotine metabolites were quantified by liquid-chromatography mass-spectrometry. The novel aspect of this study is that all isomers of 3-hydroxycotinine, with the same mass but different retention times, were examined.

Results:

- 5-hydroxycotinine and cotinine N-oxide, with their peaks well resolved from that of 3-hydroxycotinine, were detected for the first time in the plasma of nicotine-treated mice (1), in addition to the previously reported 3-hydroxycotinine. 5-hydroxycotinine was as abundant as 3-hydroxycotinine in the plasma of (i) nicotine-injected mice, and (ii) in the e-cig-treated mice with plasma cotinine levels similar to those of human smokers. Plasma cotinine-N-oxide was less abundant, but readily measurable.
- The combined plasma concentration of 3-hydroxycotinine + 5-hydroxycotinine + cotinine-N-oxide was significantly higher in female than in male mice (n=5-6), whereas for cotinine alone, the male-female difference was not significant.

Significance:

- 5-hydroxycotinine needs to be taken into account in examining nicotine disposition in vivo not only in mice, but possibly also in human smokers where its presence in the urine is controversial (2). In vitro, 5-hydroxycotinine was detected upon incubation of human CYP2A6 with hamster liver microsomes and cotinine (3). Interestingly, the approximately equal rates of 3- and 5-cotinine hydroxylation in this in vitro system are in accord with our present finding that approximately equal quantities of plasma 3-hydroxycotinine and 5-hydroxycotinine are formed by mouse CYP2A5 in vivo upon nicotine administration. 5-hydroxycotinine may have eluded detection due to glucuronidation, and/or the lower sensitivity of the previously-used tandem mass spectrometry instead of the present direct high-resolution mass-spectrometry to monitor the parent ions.
- Because the oxidation of cotinine to 3-hydroxycotinine, 5-hydroxycotinine and cotinine N-oxide is catalyzed by a single enzyme (CYP2A5), the combined plasma concentrations of these metabolites reflect better the male/-female difference in CYP2A5 activity than the concentration of cotinine alone, which is formed from nicotine by two-step reactions. Our present results strongly suggest that 5-hydroxycotinine needs to be taken into account in future studies on sexual dimorphism of CYP2A5 activity and its regulation.

References:


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