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Respiratory retention of nicotine and urinary excretion of nicotine and its five major metabolites in adult male smokers

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Abstract

Urinary excretion of nicotine and its five major metabolites (nicotine-*N*-glucuronide, cotinine, cotinine-*N*-glucuronide, *trans*-3′-hydroxycotinine, and *trans*-3′-hydroxycotinine-*O*-glucuronide), expressed as nicotine equivalents (NE), has been used as a biomarker of smoking-related nicotine exposure. In this open-label, single center study, we investigated the relationship between nicotine retention from smoking and urinary excretion of NE in adult smokers. After a 4-day washout period, 16 adult male smokers smoked 6 cigarettes per day for four consecutive days according to three predefined smoking patterns: no inhalation (Pattern A), normal inhalation (Pattern B), and deep inhalation (Pattern C). The amount of nicotine retained in the respiratory tract during smoking was estimated from the difference between the amounts of nicotine delivered and exhaled. The daily excretion of urinary NE was measured in 24 h urine samples by LC–MS/MS. The mean (±S.D.) amount of nicotine retained was 0.126 ± 0.167, 0.960 ± 0.214, and 1.070 ± 0.223 mg/cig for Patterns A, B, and C, respectively. The mean (±S.D.) relative retention (the amount retained relative to the amount delivered) was $11.2 \pm 14.7\%$, $98.0 \pm 1.6\%$, and $99.6 \pm 0.3\%$ for Patterns A, B, and C, respectively. On the fourth day of smoking, an average of $86 \pm 20\%$ of the total daily amount of retained nicotine was recovered as NE in 24 h urine. Nicotine equivalents was treated as a single component and the data was described by a first-order elimination pharmacokinetic model which assumed instantaneous input and distribution. Based on this model, the elimination half-life of NE was 19.4 ± 2.6 h, and the NE excretion had reached ∼96% of the steady state levels by Day 4. Our results suggest that most of the nicotine inhaled from a cigarette is retained (≥98%) in the lung, and at steady state, daily urine NE excretion reflects ∼90% of the retained nicotine dose from cigarette smoking.

Keywords: Nicotine; Smoking; Respiratory retention; Urinary excretion; Nicotine equivalents

1. Introduction

Nicotine (NIC) is primarily metabolized in humans to nicotine-*N*-glucuronide (NIC-G), cotinine (COT), cotinine-*N*-glucuronide (COT-G), *trans*-3′-hydroxycotinine (3HC), and *trans*-3′-hydroxycotinine-*O*-glucuronide (3HC-G). The measurement of urinary nicotine and these five major metabolites (the molar sum of the six compounds is termed as nicotine equivalents, NE) (Byrd et al., 1992; Boswell et al., 2000; Heavner et al., 2005; Roethig et al., 2005; Tricker, 2003) has been widely used as a biomarker of nicotine uptake. Previously, the extent of urinary excretion of NE in relation to the nicotine dose from smoking has been

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determined by an indirect means. A study published in 1994 (Benowitz et al., 1994) showed that ~91% of daily nicotine intake from smoking was accounted for by urinary NE. In Benowitz's study (1994), the daily nicotine intake from cigarette smoking was estimated by using metabolic clearance data obtained after intravenous infusion of isotope-labeled nicotine (d_2-nicotine) and from blood nicotine concentration data (Benowitz and Jacob, 1994). In several other studies, it was shown that NE accounted for ~85–95% of the total amount of nicotine and all measurable metabolites in urine (Tricker, 2003; Byrd et al., 1992; Andersson et al., 1997; Hecht et al., 1999; Meger et al., 2002).

The purpose of the present study was to determine the respiratory retention of nicotine while smoking cigarettes according to three predefined inhalation patterns, and to verify the relationship between the 24 h urinary NE and the nicotine dose retained from smoking.

2. Methods

2.1. Human subjects and study design

The detailed study design and subject characteristics have previously been reported (Feng et al., 2007). The protocol and informed consent form were reviewed and approved by the MDS Pharma Services Institutional Review Board. All volunteers provided written informed consent before enrolling in the study, were paid for participating, and were free to discontinue the study at any time for any reason.

Sixteen healthy adult male smokers (21–29 years old) stayed in a clinical research unit for 8 days. During the first 4 days (Day −4 to Day −1) subjects were not allowed to smoke and in the next 4 days (Day 1 to Day 4) each subject smoked six study cigarettes (a commercially available brand with 11-mg tar delivery according to the Federal Trade Commission testing method) per day at approximately 1-h intervals according to three predefined inhalation patterns. While smoking the cigarettes, each subject wore a LifeShirt® garment (VivoMetric, Ventura, CA) for respiratory monitoring. For the first two cigarettes, each subject took a puff and held the smoke in the mouth for 3 s before exhalation (Pattern A). For the third and fourth cigarettes, each subject took a puff followed by immediate normal inhalation into the lung. The subject then held his breath for 3 s before exhalation (Pattern B). For the fifth and sixth cigarettes, each subject took a puff followed by immediate deep inhalation into the lung. The subject then held his breath for 3 s before exhalation (Pattern C). There was no restriction on the puffing pattern or the number of puffs for each cigarette smoked. Each cigarette was smoked until approximately 3 mm from the tipping paper. The average inhalation volumes as measured by the LifeShirt® garment were 185 ± 171, 1081 ± 399, 2370 ± 765 mL for Patterns A, B, and C, respectively. The exhalation after each puff was directed through a mouth piece into a Cambridge Filter Pad-Fourier Transform Infrared spectrometer assembly. After smoking, the cigarette filter was analyzed for solanesol content. The same smoking procedures were repeated for 4 days, and a total of 128 measurements in 16 subjects were conducted for each predefined smoking patterns. All urine voided by each subject over a 24-h period (7:00 a.m. to 7:00 a.m.) from the first day through the end of the study, was collected and pooled into a single sample for analysis.

2.2. Respiratory retention of nicotine

As previous reported (Feng et al., 2007), we estimated the amount of nicotine delivered while smoking each individual cigarette by using a predetermined linear relationship between filter solanesol and mainstream nicotine delivery:

$$\text{Nicotine delivery} = 0.0034\,\text{Filter solanesol} + 0.0494$$

$$(R^2 = 0.876)$$

The exhaled nicotine was captured on a Cambridge Filter Pad. Filter solanesol and nicotine in exhaled breath were analyzed by HPLC and GC–MS, respectively. The amount of nicotine retained while smoking each cigarette was calculated as the difference between the amounts of nicotine delivered and exhaled:

$$\text{Amount of nicotine retained} = \text{amount delivered} - \text{amount exhaled}$$

The relative retention (expressed as a percentage value) was calculated as previously reported (Baker and Dixon, 2006):

$$\text{Relative retention (\%)} = \frac{\text{amount delivered} - \text{amount exhaled}}{\text{amount delivered}} \times 100$$

2.3. Urine nicotine and metabolites analyses

The concentrations of nicotine and five major metabolites in urine were determined by a bioanalytical method utilizing LC–MS/MS detection. Fifty microliters of aqueous solution containing internal standards of d_3-NIC, d_3-COT, d_3-3HC, d_3-NIC-G, d_3-NIC-G, d_3-COT-G, and d_3-3HC-G at 500, 500, 1000, 500, 1000, and 500 ng/mL, respectively, was added to a 0.5 mL urine sample. After adding 0.5 mL of 20 mM $HCOONH_4$ (pH 2.5), the sample was subjected to solid phase extraction on a Waters Oasis® MCX cartridge (60 mg × 3 mL) that was pre-conditioned with 2 mL of methanol and 2 mL of 20 mM $HCOONH_4$ (pH 2.5) sequentially. The sample was then eluted with 2 mL of 5% NH_4OH in methanol. The eluent was evaporated to dryness and reconstituted in 200 μL of methanol/water (70/30). Ten microliters of the extracted sample was injected into an LC–MS/MS system (AB/MDS Sciex API 4000) equipped with a guard column (Upchurch Scientific Inline filter, 0.5 μm) and analytical columns (Phenomenex Chromolith® Si, 100 mm × 4.6 mm, two columns