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Good relationship between saliva cotinine kinetics and plasma cotinine kinetics after smoking one cigarette

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ABSTRACT

This study investigated the relationship between plasma and saliva cotinine kinetics after smoking one cigarette and the relationship between cotinine kinetics and estimated nicotine intake, which was calculated as mouth level exposure (MLE) of nicotine, from smoking two test cigarettes with different nicotine yields. This study was conducted in 16 healthy adult Japanese smokers, who did not have null nor reduced-activity alleles of CYP2A6, with a quasi-randomized crossover design of smoking a low-tar cigarette or a high-tar cigarette. Saliva cotinine showed similar concentration profiles to plasma cotinine, and all of the calculated pharmacokinetic parameters of cotinine showed the same values in plasma and saliva. The C_{max} and *AUC* of cotinine showed almost the same dose-responsiveness to the estimated MLE of nicotine between plasma and saliva, but the t_{max} and $t_{1/2}$ of cotinine were not affected by the estimated MLE of nicotine in either plasma or saliva. The results show that saliva cotinine kinetics reflects plasma cotinine kinetics, and measurement of saliva cotinine concentration gives the same information as plasma cotinine on the nicotine intake. Thus, saliva cotinine would be a good and less-invasive exposure marker of cigarette smoke, reflecting the plasma cotinine concentration and kinetics.

1. Introduction

Nicotine is one of the major constituents of mainstream cigarette smoke. In humans, approximately 70–80% of absorbed nicotine is metabolized to cotinine (Hukkanen et al., 2005). The elimination half-life of cotinine is much longer than that of nicotine (Hukkanen et al., 2005), and diurnal variation of cotinine is smaller than that of nicotine in smokers. There is a good dose–response relationship of plasma cotinine to daily cigarette consumption (Benowitz et al., 2009). Based on the above, plasma cotinine has been used as an exposure marker of cigarette smoke, including subjects' smoking status (Subcommittee and on Biochemical Verification, 2002; Hatsukami et al., 2003; Benowitz et al., 2009).

There is high correlation ($r = 0.84–0.99$) between the steady–state concentrations of saliva and plasma cotinine in smokers (Parzynski et al., 2008; Jarvis et al., 2003), and saliva cotinine kinetics have been reported to be similar to plasma cotinine kinetics after intravenous and oral administrations of cotinine (Curvall et al., 1990) and when using nicotine replacement therapeutic products (NRTPs) (Rose et al., 1993). Therefore, saliva cotinine is thought to be an alternate marker of plasma cotinine and a good exposure marker of cigarette smoke without invasive methods for collecting body fluids. However, to the best of our knowledge, there are no studies examining saliva cotinine kinetics in comparison with plasma cotinine kinetics after smoking one cigarette.

Nicotine intake from smoking one cigarette varies among smokers due to differences in their smoking topography. Therefore, it is important to estimate nicotine intake by smoking topography and to examine the relationship between the estimated nicotine intake and each of plasma and saliva cotinine pharmacokinetic parameters in each subject.

The objective of this study was to investigate the relationship between plasma and saliva cotinine kinetics after smoking one cigarette. This study also included an investigation of the relationship between cotinine kinetics and estimated nicotine intake, which was calculated as mouth level exposure of nicotine by subjects' smoking topography, from smoking two test cigarettes with different International Organization for Standardization (ISO) machine-smoked nicotine yield.

Since cytochrome P450 2A6 (CYP2A6) is a major enzyme in metabolizing nicotine to cotinine, and null and reduced-activity alleles of CYP2A6 result in lower plasma cotinine concentrations (Nakajima et al., 2006; Yoshida et al., 2002; Hukkanen et al., 2005), the CYP2A6 alleles of subjects are extremely important in determining the pharmacokinetic parameters of nicotine and cotinine. Thus, to avoid complex results in pharmacokinetic parameters of nicotine and cotinine, smokers with null and reduced-activity alleles of CYP2A6 were excluded from this study.

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2. Material and methods

2.1. Subjects

16 healthy Japanese smokers (eight males and females) who were not restricted in daily cigarette consumption and its tar content, aged 21–54 years and body mass index (BMI) of 18–25 kg/m^2, were enrolled. Subjects with null or reduced-activity alleles of cytochrome P450 2A6 (CYP2A6; *1A/*7, *4/*4, *4/*7, *4/*10, *7/*7, *7/*10, *10/*10) determined by PCR (Yoshida et al., 2002) were excluded. Subjects with an average puff volume less than 35 mL, as measured by the CReSSmicro device (Plowshare Technologies, Inc./Borgwaldt KC, Inc., Richmond, USA) when smoking the test cigarettes in the same way as in the study regimen were also excluded to avoid not calculating the pharmacokinetic parameters by one LTC smoking. Pregnant or breast-feeding females were excluded from this study. Before entering the trial, the health conditions of subjects were confirmed by physical examination, medical history, vital signs, 12-lead electrocardiogram, laboratory tests and a respiratory function test, and a pregnancy test for female subjects. Additionally, any subjects having serum cotinine concentrations over 42 ng/mL, as determined using an EIA kit (OraSure Technologies, Inc., Pennsylvania, USA) on each day prior to smoking the test cigarette, were also excluded. Analysis of CYP2A6 polymorphisms was conducted by Mitsubishi Chemical Medience Corporation (Tokyo, Japan), and information on subject's CYP2A6 polymorphisms was used for screening. However, such information was not disclosed to the sponsor. All subjects provided written informed consent before enrollment in this study.

2.2. Study design

This study was a single-dose, quasi-randomized crossover study to investigate the kinetics of nicotine and cotinine after smoking a low-tar commercially available cigarette (LTC: 1 mg tar and 0.1 mg nicotine yield by ISO machine-smoking (ISO 3308, 2000)) and a high-tar commercially available cigarette (HTC: 10 mg tar and 0.8 mg nicotine yield). This study was comprised of two periods, each of which consisted of seven days. Subjects were quasi-randomly assigned to one of two sequences of a gender-balanced crossover design in the order of visiting at screening, and smoked only one test cigarette, HTC or LTC, in each period. Subjects abstained from smoking for 96 h before smoking the test cigarette and for 72 h after smoking in each period. Additionally, subjects stayed in the medical institution for four days from 24 h before smoking the test cigarette. Smoking for one test cigarette was set as one puff per 10 s, with a total of 12 puffs, and it was completed within 2 min. In order to obtain the pharmacokinetic parameters even at smoking one LTC, this short term smoking duration was set.

Blood samples were collected just before and at 2, 5, 10, 15, 20, 25, 30, 45, and 60 min and 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 12, 24, 48, and 72 h after smoking the test cigarette. Blood samples were collected in EDTA-2Na containing blood collection tubes, and then centrifuged at 2000×*g* for 10 min at 4 °C. Plasma samples were transferred into plastic tubes and stored at −80 °C until analysis. Saliva samples were collected via a straw by placing sour food (lemons) in front of subjects, just before and at 1, 2, 3, 12, 24, 48, and 72 h after smoking the test cigarette. Saliva samples were transferred into a plastic tube and stored at −80 °C until analysis.

This study was conducted at an external medical institution for clinical research in Japan in accordance with Good Clinical Practice and the Declaration of Helsinki. This study protocol was approved by the Institutional Review Boards of Japan Tobacco Inc. and the external medical institution.

2.3. Bioanalysis

2.3.1. Analyses of plasma nicotine and cotinine

A 25 μL of distilled water, 500 μL of 0.028% aqueous ammonia and 25 μL of methanol solution containing nicotine-d_3 and cotinine-d_3 (80 ng/mL each) as internal standards were added to 200 μL of the plasma sample. The sample components were extracted by solid phase extraction with Empore™ universal resin plate (Sumitomo 3 M Ltd., Tokyo, Japan), then eluted with 200 μL of methanol. A 10 μL aliquot of the eluate was analyzed using a liquid chromatography (1100 system, Agilent Technology, Inc., California, USA)/tandem mass spectrometry (Quattro Ultima™, Waters Corp., Massachusetts, USA) system. Chromatography was performed on a column (Inertsil Ph-3, 5 μm particle size, 150 mm × 2.1 mm id, GL Sciences Inc., Tokyo, Japan) with a mobile phase consisting of aqueous ammonium acetate (0.01 M) containing 0.01% acetic acid–methanol-acetonitrile (60:20:20). Nicotine and cotinine in the eluted fraction were identified by their respective retention times and by using a precursor/fragment ion with electrospray ionization (ESI) and selected reaction monitoring (SRM) method. The calibration plots were prepared using blank human plasma spiked with standard substances at seven concentrations, ranging from 0.5 to 200 ng/mL. Calibration curves were constructed from the peak area ratios of standard substances to their internal standards vs. concentrations using a 1/Y weighted linear least-square regression method. The lower limits of quantification (LLOQ) for both nicotine and cotinine were 0.5 ng/mL. Analyses were conducted by Mitsubishi Chemical Medience Corporation.

2.3.2. Analyses of saliva nicotine and cotinine

A 1275 μL of distilled water and 25 μL of methanol solution containing nicotine-d_3 and cotinine-d_3 (20 ng/mL each) as internal standards were added to 50 μL of the saliva sample, shaken for 30 s and then centrifuged at 17,000×*g* for 10 min at 4 °C. One mL aliquot of the supernatant was analyzed using a liquid chromatography (2795 system, Waters Corp., Massachusetts, USA)/tandem mass spectrometry (Finnigan TSQ Quantum, Thermo Fisher Scientific Inc., Massachusetts, USA) system on columns (CAPCELL PAK C18 MGII, 5 μm particle size, 100 mm × 2.0 mm id, Shiseido Co., Ltd., Tokyo+Discovery HS F5, 5 μm particle size, 50 mm × 2.1 mm id, Sigma–Aldrich Inc., Missouri, USA) with a stepwise gradient and a mobile phase consisting of aqueous ammonium hydrogen carbonate (5 mM)-methanol (100:0, 20:80, 100:0). Additionally, foreign substances were removed by back-flushed liquid chromatography (2690 system, Waters Corp., Massachusetts, USA) on a guard column (GUARD CARTRIDGE CAPCELL PAK C18 MGII, 5 μm particle size, 10 mm × 4.0 mm id, Shiseido Co., Ltd., Tokyo, Japan) with a mobile phase consisting of aqueous ammonium acetate (0.01 M) containing acetic acid (1000:0.1)-methanol (75:25) using a column-switching method. Nicotine and cotinine in the eluted fraction were identified by their respective retention times and a precursor/fragment ion method with ESI and SRM. The calibration plots for nicotine were prepared using blank human saliva spiked with standard substances at six concentrations, ranging from 10 to 400 ng/mL. The calibration plots for cotinine were prepared using blank human saliva spiked with standard substances at eight concentrations, ranging from 1 to 400 ng/mL. Calibration curves were constructed from the peak area ratios of standard substances to their internal standards vs. concentrations using a 1/Y weighted linear least-square regression method. The LLOQ for nicotine was 10 ng/mL and that for cotinine was 1 ng/mL. Analyses were also conducted by Mitsubishi Chemical Medience Corporation.