Drug Metab. Pharmacokinet. 26 (4): 416–422 (2011).

Regular Article

Pharmacokinetic Study of Nicotine and Its Metabolite Cotinine to Clarify Possible Association between Smoking and Voiding Dysfunction in Rats Using UPLC/ESI-MS

Satomi ONOUE*, Noriyuki YAMAMOTO, Yoshiki SETO and Shizuo YAMADA

Department of Pharmacokinetics and Pharmacodynamics and Global Center of Excellence (COE) Program, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: The present study was undertaken to clarify the possible association between nicotine intake/cigarette smoking and detrusor instability. For pharmacokinetic characterization of nicotine and cotinine (a major and pharmacologically less active metabolite of nicotine), a rapid ultra-performance liquid chromatography/electrospray ionization-mass spectrometry (UPLC/ESI-MS) method was developed that requires only a small amount of sample and simple pretreatment. The UPLC/ESI-MS method was validated with a focus on specificity, sensitivity (limit of detection, 2.5 ng/mL; limit of quantification, 5 ng/mL), linearity ($r > 0.998$), accuracy (97.2–102.8%), precision (relative standard deviation <8%) and robustness in accordance with ICH guidelines (Q2B Validation of Analytical Procedures: Methodology). The developed method was successfully applied to determine nicotine and cotinine levels in rat biological samples such as plasma, urine and several tissues. After subcutaneous administration of nicotine ditartrate (2 mg/kg of body weight) in rats, the absorbed nicotine was rapidly and extensively metabolized into cotinine. However, nicotine was found to be predominant in cortex and bladder, where nicotinic acetylcholine receptors were expressed for neuronal control of voiding function. Repeated administration of nicotine led to a ca. 3-fold higher accumulation of nicotine than that of cotinine in rat urine. The results of the pharmacokinetic study using the UPLC/ESI-MS method further support the possible involvement of nicotine in increased risk of urinary dysfunction in smokers.

Keywords: nicotine; cotinine; UPLC; urinary excretion; cigarette smoking

Introduction

Cigarette smoke is a complex aerosol consisting of 92% gaseous components and 8% particulates, and side stream and direct stream smoke are similar in composition.[1)] The inhalation of cigarette smoke either directly by cigarette smokers or indirectly by people in the same enclosed areas represents an environmental health problem for millions of people.[2)] Cigarette smoking has long been identified as a major causative factor in the development of inflammatory lung diseases such as chronic bronchitis, pulmonary fibrosis, emphysema and chronic obstructive pulmonary disease.[3)] In addition to these respiratory diseases, cigarette smoking has also been causally linked with obesity, osteoporotic fractures,[4)] cardiovascular diseases,[5)] neurodegenerative diseases[6)] and bladder dysfunctions.[7)] In particular, recent attention has been drawn to the increased risk of urinary dysfunction in female smokers.[8)] Previous population-based studies also demonstrated cigarette smoking to be associated with both urinary incontinence[7)] and urgency in elderly people,[9)] although the evidence was conflicting. In general, several factors such as weakening of the pelvic floor muscles and collagen tissue alterations through aging and childbirth may contribute to both conditions, and these urinary dysfunctions have a substantial influence on quality of life and activities of daily living.

Although little is known about the pathogenesis of smoking-related voiding dysfunction, detrusor instability in female smokers may be related to an anti-estrogenic hormonal effect on the bladder or urethra, an adverse effect

Received; March 8, 2011, Accepted; April 26, 2011
J-STAGE Advance Published Date: May 12, 2011, doi:10.2133/dmpk.DMPK-11-RG-019
**To whom correspondence should be addressed:* Dr. Satomi ONOUE, *Department of Pharmacokinetics and Pharmacodynamics and Global Center of Excellence (COE) Program, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan. Tel. +81-54-264-5633, Fax. +81-54-264-5635, E-mail: onoue@u-shizuoka-ken.ac.jp*

on collagen synthesis[10] and an increased turnover of vitamin C.[11] It is also well established that nicotine plays an important role in the control of bladder function, mediated by nicotinic acetylcholine receptors in both central and peripheral nervous systems.[12–14] In this context, nicotine in cigarette smoke might also lead to detrusor instability *via* neuronal control of urinary bladder responses.[15] Better understanding of the pharmacokinetic behavior of nicotine may identify the involvement of nicotine in detrusor instability in smokers; however, it has never been fully elucidated.

Many analytical methods have been developed for the analysis of nicotine and its metabolites, including gas chromatography,[16] high-performance liquid chromatography (HPLC),[17,18] enzyme-linked immunosorbent assay (ELISA)[19] and radio-immunoassay (RIA).[20] The present study was undertaken to develop a more rapid analytical method for pharmacokinetic characterization of nicotine and cotinine, a major metabolite of nicotine, using ultra-performance liquid chromatography (UPLC)/electrospray ionization (ESI)-MS. The UPLC/ESI-MS method for nicotine was validated with a focus on linearity, accuracy, precision, assay recovery and robustness. Plasma and urine concentrations of nicotine and cotinine after subcutaneous injection were monitored using the developed UPLC/ESI-MS method, and the tissue distribution of these chemicals was also assessed.

Materials and Methods

Chemicals: Ammonium acetate, nicotine ditartrate and diethyl ether were purchased from Wako Pure Chemical Industries (Osaka, Japan). Cotinine, antipyrine and sodium pentobarbital were purchased from Sigma (St. Louis, MO, USA). Acetonitrile (HPLC grade) and Millex-LG membrane filters (pore size: 0.2 μm) were bought from Kanto Chemical (Tokyo, Japan) and Millipore (Bedford, MA, USA), respectively.

UPLC/ESI-MS analysis: The amounts of nicotine and cotinine in biological samples from rats were determined by an internal standard method using a Waters Acquity UPLC system (Waters, Milford, MA), which included a binary solvent manager, sample manager, column compartment and SQD connected with MassLynx software. An Acquity UPLC BEH C 18 column (particle size: 1.7 μm, column size: 2.1 mm × 50 mm; Waters) was used, and the column temperature was maintained at 40°C. The standard and samples were separated using a gradient mobile phase consisting of acetonitrile (A) and 5 mM ammonium acetate (B) with a flow rate of 0.25 mL/min. The gradient condition of the mobile phase was 0–0.5 min, 5% A; 0.5–3.5 min, 5–25% A; 3.51–4.5 min, 95% A; and 4.51–5.5 min, 5% A. Analysis was carried out using selected ion recording (SIR) for m/z 163 for nicotine $[M + H]^+$, 177 for cotinine $[M + H]^+$ and 189 for antipyrine $[M + H]^+$, an internal standard. Peaks for nicotine, cotinine and antipyrine were detected at retention times of 1.90, 2.30 and 3.26 min, respectively.

Method validation: The newly developed UPLC/ESI-MS method was validated in terms of linearity, accuracy, precision and assay recovery according to International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines "Q2B Validation of Analytical Procedures: Methodology". Assay method precision was investigated using six independent test solutions and a standard preparation. The intermediate precision of the assay was also evaluated using different analysts on three different days. The accuracy of the assay was evaluated in triplicate using three concentration levels: 10, 300 and 600 ng/mL. The limit of detection (LOD) and limit of quantification (LOQ) for nicotine and cotinine were estimated by injecting a series of dilute solutions with known concentration. The LOD and LOQ were estimated from standard deviation values of replicate responses of tested chemicals (signal-to-noise ratios, 3:1 for LOD and 10:1 for LOQ). To determine the robustness of the method, experimental conditions were purposely altered and the peak response for each tested chemicals was examined by injecting system suitability solution. The flow rate was changed to 0.20 and 0.30 mL/min. The column temperature was varied by (±)3°C, and the organic strength was varied by (±)2%.

Pharmacokinetic study:

Animals

Male Sprague–Dawley rats, weighing ca. 650 g ($n = 4$ for each *in vivo* experiment, 25–30 weeks of age; Japan SLC, Shizuoka, Japan), were housed two per cage in the laboratory with free access to food and water, and maintained on a 12-h dark/light cycle in a room with controlled temperature (24 ± 1°C) and humidity (55 ± 5%). All procedures used in the present study were conducted in accordance with the guidelines approved by the Institutional Animal Care and Ethical Committee of the University of Shizuoka.

Plasma concentration

Rats were anesthetized using pentobarbital (50 mg/kg body weight) and then a guide cannula (PUC-40, EICOM, Kyoto, Japan) was inserted into the jugular vein on the day before nicotine ditartrate (2 mg/kg body weight) was administered subcutaneously. Blood samples (approximately 500 μL) were collected from the cannulated jugular vein at the indicated times (0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 10 and 12 h) after administration of nicotine ditartrate and mixed with 1,250 μL of acetonitrile containing 0.053 nM antipyrine. The mixture was centrifuged (10,000 × g, 10 min, 4°C) and the supernatant was dried with a stream of nitrogen gas. Each dried sample was redissolved in 100 μL of 50% acetonitrile solution and filtered with Millex-LG membrane filter (Millipore, Bedford, MA, USA) for UPLC/ESI-MS analysis.

Urine concentration

After single subcutaneous administration of nicotine ditartrate (2 mg/kg body weight), urine samples were