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Original research article

Pharmacokinetics of cotinine in rats: A potential therapeutic agent for disorders of cognitive function

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ABSTRACT

Background: Attention has been paid to cotinine (COT), one of the major metabolites of nicotine (NIC), for its pro-cognitive effects and potential therapeutic activities against Alzheimer's disease (AD) and other types of cognitive impairment. In order to facilitate pharmacological and toxicological studies on COT for its pro-cognitive activities, we conducted a pharmacokinetic (PK) study of COT in rats, providing important oral and intravenously (*iv*) PK information.

Methods: In this study, plasma samples were obtained up to 48 h after COT was dosed to rats orally and *iv* at a dose of 3 mg/kg. Plasma samples were prepared and analyzed using a sensitive liquid chromatography tandem mass spectrometry (LC–MS/MS) bioanalytical method, providing concentration profiles of COT and metabolites after oral and *iv* administrations.

Results: The data were fitted into a one-compartment model and a two-compartment model for the oral and *iv* groups, respectively, providing important PK information for COT including PK profiles, half-life, clearance and bioavailability. The results suggested fast absorption, slow elimination and high bioavailability of COT in rats.

Conclusions: Several important facts about the PK properties in rats suggested COT could be a potential pro-cognitive agent. Information about the pharmacokinetics of COT in rats revealed in this study is of great importance for the future studies on COT or potential COT analogs as agents for improving cognition.

Introduction

As a result of the unprecedented growth of elderly populations, one of the most significant results of global population aging is the rise in the number of people suffering from age-related forms of dementia, including Alzheimer's disease (AD), schizophrenia, Parkinson's disease and mild cognitive impairment (MCI) [1]. AD is a progressive, severe and incurable neurodegenerative disorder, of which the actual cause and pathological mechanism of AD are still not clearly known [2]. Different theories have been published as the possible mechanisms of AD, in which the most accepted one is related to the aggregation of amyloid-β (Aβ) peptides, amyloid angiopathy, and neurofibrillary tangles of phosphorylated tau protein in the brain [3–5]. Current therapeutic agents for AD, including memantine, galantamine and xanomeline can only alleviate the symptoms and may cause significant side effects [6–9]. Similarly, limited therapeutic agents are available for the effective and safe treatment of schizophrenia, Parkinson's disease and MCI.

The pro-cognitive effects of tobacco have been of great interest to researchers in the past decades [10–12]. Nicotine (NIC) has been demonstrated to have pro-cognitive effects on the central nervous system by acting as an agonist for nicotinic acetylcholine receptors (nAChRs) [13,14]. However, due to the short half-life (2–6 h), high toxicity (mouse oral LD50 = 50 mg/kg) and high addictive potential of NIC, it is unlikely to be developed as an effective and safe therapeutic agent. The major metabolite of NIC, cotinine (COT), has shown pro-cognitive effects in animals [15–17]. COT is a weak agonist of nAChRs, however, its mechanism of action for the pro-cognitive effects is still yet to be fully elucidated [18]. COT was reported to reduce amyloid-β aggregation and improve memory in AD animal models [19]. A recent study published by Gao et al. also reported that COT demonstrated neuroprotective effects, which could also contribute to the prevention and treatment of

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AD [20]. COT also showed significant pro-cognitive effects by attenuating glutamate (NMDA) antagonist-related effects in an animal behavioral study published by Terry et al., which was considered to be valuable for the treatment of schizophrenia [17]. In addition, a study on primate species revealed that COT could selectively activate some nicotinic receptors and showed activities that could be used for the treatment of Parkinson's disease [21]. Last but not least, the longer biological half-life (15–19 h) and lower toxicity (mouse oral LD50 = 1604 mg/kg) of COT make it a more practical prototype drug candidate for the treatment of AD and other mild to severe forms of dementia.

Several studies have been conducted to reveal the pharmacokinetic (PK) properties of NIC [22–25]. NIC was reported to have a low oral factor of bioavailability (about 20%) and short half-life (2–6 h) by these studies. A few PK properties of COT were revealed from some PK studies focused on NIC, providing limited information about the half-life and clearance of COT as a metabolite of NIC [23,26]. Two studies about the PK of COT in humans were published in 1987 and 1990, respectively, revealing important PK parameters of COT in non-smoking healthy volunteers [27,28].

However, no PK studies have been conducted on rodents. Rodents are frequently used as animal models in non-clinical and pre-clinical drug research and development. In most of the current pre-clinical studies on the pre-cognitive effects of COT, rodents are used as test animals for experiments on behavior, pharmacology and toxicology. Information and conclusions about the pro-cognitive effects of COT are primarily based on rodents. Therefore, PK profiles of COT in rodent species are needed as guidance for the administration of COT in future studies. It is also of great important to have the PK properties of COT available, which could be correlated to the brain distribution and pharmacological activities of COT. Moreover, in humans, COT is primarily metabolized by cytochrome P450 2A6 (CYP2A6), which does not exist in rodents including mice and rats [29]. Therefore, COT may display different metabolism, disposition and PK in rodent species. A PK study of COT in rodents is needed to obtain important PK parameters for future non-clinical and pre-clinical studies on the pharmacology, toxicology and drug delivery of COT. With the PK properties of COT in rats available, such studies can be more specifically conducted, which could be the foundation for further investigations into the pro-cognitive effects of COT on humans.

In this study, we used a sensitive, precise and accurate LC–MS/MS method for the quantification of COT and three other major metabolites in rat plasma [30]. Test rats were dosed with a single dose of COT at 3 mg/kg both orally and intravenously, which was the therapeutic dosage level for pro-cognitive effects. Important PK information of COT including PK profiles, half-life, clearance and bioavailability were revealed in this study, which suggested fast absorption, slow elimination and high bioavailability of COT in rats. Moreover, three major metabolites of COT, norcotinine (NCOT), *trans*-3′-hydroxcotinine (OHCOT) and (*S*)-cotinine-N-oxide were also analyzed simultaneously, providing more information about the bio-transformation of COT.

Though much information about the *in vivo* effects of cotinine is obtained from rodent models, this is the first manuscript designed to specifically focus on cotinine PK in rats. These results about the PK of COT in rats are of great importance for future studies on COT and its pro-cognitive effects.

Experimental

Chemicals and reagents

(−)-Cotinine (COT) was purchase from Sigma–Aldrich (St. Louis, MO, USA). Stable isotope labeled internal standard (IS) (±)-cotinine-d3 solution (1 mg/mL in methanol) was obtained from Cerilliant (Round Rock, TX). (*R*,*S*)-norcotinine (NCOT), *trans*-3′-hydroxcotinine (OHCOT) and (*S*)-cotinine-N-oxide, (*R*,*S*)-norcotinine-d4 (NCOT-d4), *trans*-3′-hydroxycotinine-d3 (OHCOT-d3) and (*R*,*S*)-cotinine-N-oxide-d3 (COTNO-d3) were purchased from Toronto Research Chemical (Toronto, Canada). Chemical structures of COT, NCOT, OHCOT and COTNO are shown in Fig. 1. Trichloroacetic acid and ammonium acetate were obtained from Baker (Phillipsburg, NJ, USA). LC–MS grade formic acid, acetonitrile (ACN), methanol and water were from Sigma (St. Louis, MO, USA).

Solutions and standards

Individual stock solutions of all the analytes and IS were prepared as 1.0 mg/mL methanol solutions. Combined working solutions were obtained by serial dilution with 90% ACN/water (v/v, 9/1). IS working solutions containing COT-d3, NCOT-d4, OHCOT-d3 and COTNO-d3 were prepared at a single concentration of 500.0 ng/mL in the same solvent. Stock solutions were kept at −20 °C when not in use.

Blank rat plasma with sodium EDTA was purchased from Bioreclamation (Westbury, NY). 10 μL of standard or QC working solution was spiked into 100 μL of blank plasma to generate corresponding standard or QC samples. The final concentrations of calibration standards were 20, 50, 100, 200, 500, 1000, 5000 and 10,000 ng/mL in plasma while the QC samples were 30, 750 and 7500 ng/mL. Fresh standards and QC samples were prepared on the day of experiments.

Dosing and sample collection

Pre-canulated albino Wistar rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN, USA) approximately 2 months old were housed in pairs in a temperature controlled room (25 °C), maintained on a 12:12 h normal light–dark cycle (lights on at 6 AM) with free access to water and food until used for PK studies. All procedures employed during this study were reviewed and approved by the Georgia Regents University Institutional Animal Care and Use Committee and are consistent with AAALAC guidelines. Measures were taken to minimize pain and discomfort in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996.

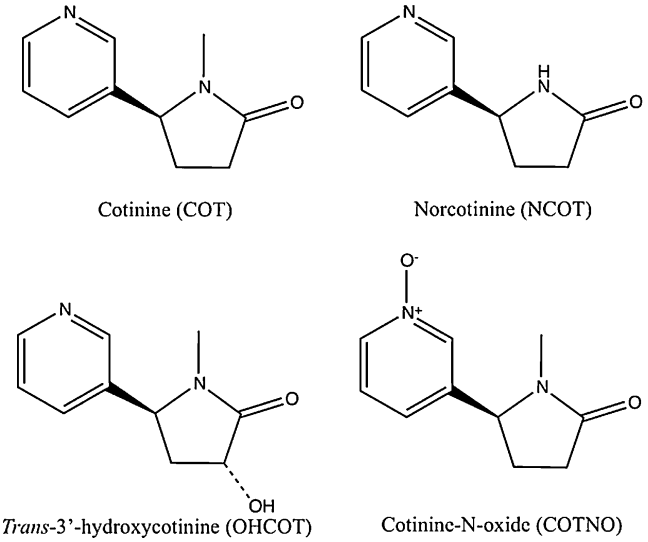

Fig. 1. Chemical structures of cotinine (COT), norcotinine (NCOT), *trans*-3′-hydroxylcotinine (OHCOT) and cotinine-N-oxide (COTNO).