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Application of a pharmacokinetic/pharmacogenetic approach to assess the nicotine metabolic profile of smokers in the real-life setting

Stefano Fogli[a,*], Alessandro Saba[b], Marzia Del Re[c], Francesco Pistelli[d], Ferruccio Aquilini[d], Riccardo Zucchi[c], Antonio Palla[b,d], Maria Cristina Breschi[a], Laura Carrozzi[b,d], Romano Danesi[c]

[a] Department of Pharmacy, University of Pisa, Pisa, Italy
[b] Department of Pathology, University of Pisa, Pisa, Italy
[c] Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy
[d] Cardio-Thoracic and Vascular Department, University Hospital of Pisa, Pisa, Italy

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ABSTRACT

The nicotine metabolite ratio, i.e., the ratio 3-hydroxycotinine/cotinine, is used to assess the nicotine metabolic status and has been proven to predict the response to smoking cessation treatments in randomized clinical trials. In the current study, a pharmacokinetic-pharmacogenetic integrated approach is described, based on the development of a liquid chromatography–tandem mass spectrometry (LC/MS/MS) method for nicotine metabolite ratio assay in plasma and a real-time PCR analysis for fast genotyping of CYP2A6. The pharmacokinetic-pharmacogenetic approach was validated in 66 subjects with different smoking status. The LC/MS/MS assay was rapid and sensitive enough to detect plasma cotinine levels also in second-hand exposed abstainers. In the cohort of patients of the present study the following results were obtained: (i) the frequencies of CYP2A6 genetic variants were comparable with those from clinical trials carried out in Caucasian populations; (ii) all the subjects carrying the CYP2A6 deficient allele also had a slow metabolizer phenotype; (iii) slow metabolizers had mean nicotine metabolite ratio approximately 50% of that of the normal/fast metabolizers; (iv) women had higher nicotine metabolite ratio than men; and (v) salivary nicotine metabolite ratio measures were comparable to plasma levels. Overall, the findings of the current study demonstrate that the simultaneous assessment of nicotine metabolite ratio and CYP2A6 genotype from human blood samples is feasible and accurate and could be used in a smoking cessation program to optimize treatments and identify those smokers who inherit metabolically deficient CYP2A6 alleles.

1. Introduction

Nicotine dependence is recognized as a medical condition and active and secondhand smoking have been associated to a myriad of human diseases [1]. For these reasons, smoking cessation is a prominent health issue; however, despite the efficacy of currently available pharmacotherapy, only 5–35% of treated subjects continue to abstain from smoking [2]. One possible explanation of the high percentage of treatment failures is the inter-individual variability of nicotine metabolism that may cause inadequate drug exposure after transdermal nicotine replacement therapy (tNRT) in combination with bupropion and varenicline [3].

Nicotine is metabolized to its major active metabolite cotinine (COT) by the hepatic enzyme CYP2A6; COT is subsequently converted into *trans*-3-hydroxycotinine (3HC), almost exclusively by CYP2A6 [4]. Several genetic variants of CYP2A6 have been identified and associated with inter-individual variability in nicotine metabolism with effects on smoking behaviour [5]. CYP2A6 genotypes display different frequencies among ethnic groups [6]. A prospective nicotine patch trial was aimed to stratify smokers on the basis of serum COT levels. It was demonstrated that smokers with low baseline COT plasma concentrations (<250 ng/ml) responded better to tNRT than those with high COT levels [7].

* Corresponding author at: Department of Pharmacy, University of Pisa, Via Bonanno, 6, 56126 Pisa, Italy.
E-mail address: stefano.fogli@farm.unipi.it (S. Fogli).

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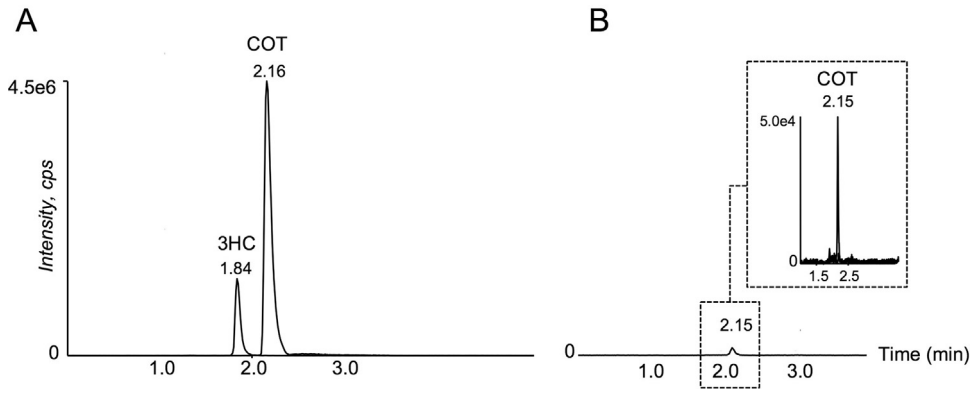

Fig. 1. Real sample chromatograms from (A) a smoker who smoked 18 cig/day, and (B) an ex-smoker, abstinent for 5 months. COT: cotinine; 3HC: 3-hydroxycotinine.

These results have been subsequently confirmed by others using the plasma 3HC/COT ratio (also termed as "nicotine metabolite ratio"), as a phenotypic marker for CYP2A6 activity that correlates with nicotine metabolic clearance [8–10]. Specifically, slow metabolizers (baseline mean 3HC/COT ratio < or = 0.26) were considered good candidates for standard tNRT, whereas fast metabolizers could instead benefit from bupropion [10], which is not a substrate of CYP2A6. More recently, a randomized, double blind, placebo-controlled clinical trial demonstrated the usefulness of a nicotine metabolite ratio cut-off of 0.31 to guide treatment decision between nicotine patch or varenicline [11].

It is worth mentioning that the results of this type of studies cannot always be translated into the real-word setting due to a number of factors, including strict patient inclusion criteria, standardized treatments and patient compliance. For these reasons, it is important to validate robust biomarkers of smoke exposures. The current study describes a pharmacokinetic-pharmacogenetic integrated approach to accurately assess the nicotine metabolic profile of smokers in the real-life setting. These findings could be easily translated into clinical recommendations within smoking cessation programs and could help physicians to select the most appropriate drug for each smoker.

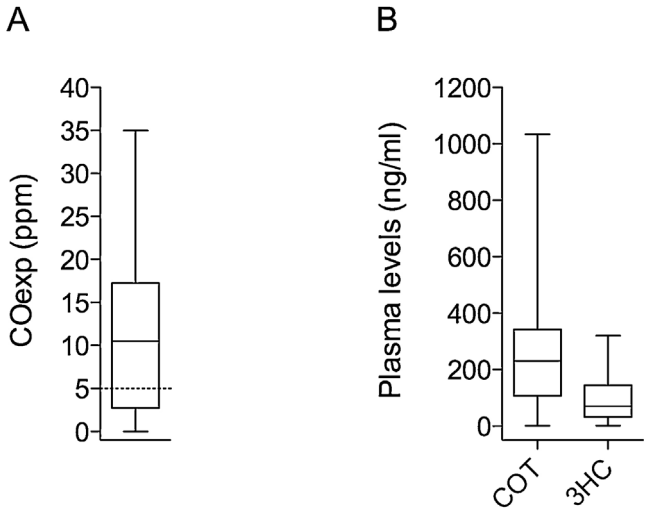

Fig. 2. (A) Distribution of carbon monoxide level in expired air (COexp) and (B) plasma levels of cotinine (COT) and *trans*-3-hydroxycotinine (3HC) in study population.

2. Materials and methods

2.1. Subjects and samples collection

The study was submitted and approved by the Ethics Committee of Pisa University Hospital and conducted in accordance to the principles of the Declaration of Helsinki. All subjects gave their signed informed consent before their blood samples were obtained.

Participants were 66 subjects attending the Smoking Cessation Centre of the University Hospital (Pisa, Italy), treated as per current clinical practicewith nicotine replacement therapy NRT – as transdermal patches, lozenges, inhalers or gums, varenicline or bupropion. The following clinical informations were collected by administering standard questionnaires: smoking status (including date and hour of the last cigarette/cigar smoked; number of cigarettes smoked from awakening in the day of the blood sampling; number of cigarettes smoked per day in the last 7 days); level of nicotine dependence (according to Fagerström Test for Nicotine Dependence score); number of pack-years; use of electronic cigarettes (E-cig); health status; current use of pharmacotherapy for smoking cessation (including NRT or other medicines).

Paired plasma and saliva samples were obtained from a subset of participants (n = 9) to confirm whether saliva could be a used to reliably assess the nicotine metabolite ratio. An aliquot of blood samples was centrifuged at 1,000 × *g* for 10 min to obtain plasma; plasma and whole blood were stored at 4 °C and used within 72 h to measure plasma levels of COT and 3HC and analyze CYP2A6 polymorphisms, respectively. For saliva collection, the Salivette® device (Sarsted AG & Co., Nümbrecht, Germany) was used. Saliva samples were centrifuged for 2 min at 1,000 × *g* to yield clear supernatant in a conical tube and stored as for blood samples. An heterogeneous sample of subjects was enrolled, regardless of the pharmacological treatment, health or smoking status (i.e., current smokers or abstainers). Cigarette or cigar tobacco smokers and/or E-cig users were enrolled. Carbon monoxide level in expired air (CO_{exp}) was measured after a deep inspiration and an apnoea of 10 s by using the Mycro 4 Smokerlyzer (Bedfont Scientific Ltd, Rochester, England). "Abstainers" were defined as those subjects who reported not having smoked tobacco cigarettes or cigars in the last seven days and had a CO_{exp} < 5 ppm.

2.2. LC/MS/MS analyses and definition of nicotine metabolic ratio

Stock solutions of COT and 3HC, and their internal standards, COT-D3 and 3HC-D3, were prepared at a concentration of 1 mg/ml in methanol and stored at −20 °C. Sample preparation was performed as follows: 100 μl of plasma or saliva and 300 μl acetonitrile