POPULATION PHARMACOKINETICS OF NICOTINE IN THAI SMOKERS

Miss Kathy Moe San

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy in Clinical Pharmacy
Department of Pharmacy Practice
Faculty of Pharmaceutical Sciences
Chulalongkorn University
Academic Year 2019

เภสัชจลนศาสตร์ประชากรของนิโคตินในผู้สูบบุหรี่ชาวไทย

น.ส.เคธี่ โม แซน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชกรรมคลินิก ภาควิชาเภสัชกรรมปฏิบัติ
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2562

Thesis Title	POPULATION PHARMACOKINETICS OF NICOTINE IN THAI SMOKERS
By	Miss Kathy Moe San
Field of Study	Clinical Pharmacy
Thesis Advisor	Assistant Professor Thitima Wattanavijitkul, Ph.D.
Thesis Co Advisor	Assistant Professor PAJAREE CHARIYAVILASKUL, Ph.D.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science in Pharmacy

..... Dean of the Faculty of Pharmaceutical Sciences

(Assistant Professor RUNGPETCH SAKULBUMRUNGSIL, Ph.D.)

THESIS COMMITTEE

..... Chairman

(Associate Professor WANCHAI TREYAPRASERT, Ph.D.)

..... Thesis Advisor

(Assistant Professor Thitima Wattanavijitkul, Ph.D.)

..... Thesis Co-Advisor

(Assistant Professor PAJAREE CHARIYAVILASKUL, Ph.D.)

..... Examiner

(Tatta Sriboonruang, Ph.D.)

..... External Examiner

(Richard Hoglund, Ph.D.)

เคธี่ โม แซน : เภสัชจลนศาสตร์ประชากรของนิโคตินในผู้สูบบุหรี่ชาวไทย. (POPULATION PHARMACOKINETICS OF NICOTINE IN THAI SMOKERS) อ.ที่ปรึกษาหลัก : ผศ. ภญ. ดร.ธิติมา วัฒนวิจิตรกุล, อ.ที่ปรึกษาร่วม : ผศ. พญ. ปาจรีย์ จริยวิลาศกุล

แม้ว่าหมากฝรั่งนิโคตินเป็นที่นิยมในประเทศไทย การศึกษาเภสัชจลนศาสตร์ประชากรของหมากฝรั่งนิโคตินในชาวไทยยังไม่เคยมีการศึกษามาก่อน การศึกษานี้จึงมีวัตถุประสงค์เพื่อสร้างแบบจำลองทางเภสัชจลนศาสตร์ประชากรของหมากฝรั่งนิโคติน เพื่อศึกษาผลของปัจจัยทางพันธุกรรม และ ปัจจัยอื่น ๆ ต่อเภสัชจลนศาสตร์ของนิโคตินหลังผู้สูบบุหรี่ชาวไทยได้รับหมากฝรั่งนิโคติน การศึกษานี้วิเคราะห์ ข้อมูลทุติยภูมิจากการศึกษาทางคลินิกก่อนหน้าซึ่งศึกษาจีโนไทป์ของ cytochrome P450 2A6 (CYP2A6) ในผู้สูบบุหรี่ชาวไทย อาสาสมัครผู้สูบบุหรี่สุขภาพดีจำนวน 18 ราย ได้รับหมากฝรั่งนิโคตินขนาด 2 mg เก็บตัวอย่างเลือดที่เวลาก่อนได้รับหมากฝรั่งและที่ 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4.5, และ 6 ชั่วโมงหลังได้รับหมากฝรั่ง วิเคราะห์แบบจำลองทางเภสัชจลนศาสตร์โดยวิธี nonlinear mixed effect modeling แบบจำลองชนิดหนึ่งห้องที่มีการดูดซึมยาแบบปฏิกิริยาอันดับหนึ่งที่มีแบบจำลองหน่วยย่อยชนิดทรานซิสหกห้องและการกำจัดยาแบบปฏิกิริยาอันดับหนึ่งสามารถอธิบายข้อมูลได้ดีที่สุด การทำงาน ของเอนไซม์ CYP2A6 ในจีโนไทป์ที่แตกต่างกันเป็นปัจจัยที่มีผลต่อค่าอัตราการกำจัดยานิโคตินอย่างมีนัยสำคัญ ค่าเฉลี่ยของ apparent clearance (CL/F) และ apparent volume of distribution (V/F) ในผู้สูบบุหรี่ที่มีการทำงานของจีโนไทป์ CYP2A6 ปกติ (หรือมี การทำงานของเอนไซม์ CYP2A6 เป็นร้อยละ 100) มีค่าเท่ากับ 266 ลิตร/ชั่วโมง และ 851 ลิตร ตามลำดับ ผลการตรวจสอบความถูกต้องของแบบจำลองด้วยวิธี Bootstrap และวิธี Visual predictive check จำนวน 1,000 ครั้ง แสดงว่าแบบจำลองมีความถูกต้องและเหมาะสม งานวิจัยนี้เป็นงานวิจัยแรกที่สร้างแบบจำลองเภสัชจลนศาสตร์ประชากรของหมากฝรั่งนิโคตินในอาสาสมัครชาวไทย และหาค่า CL/F ของนิโคตินในผู้สูบบุหรี่ที่มีจีโนไทป์ของ CYP2A6 แตกต่างกัน

สาขาวิชา	เภสัชกรรมคลินิก	ลายมือชื่อนิสิต .....
ปีการศึกษา	2562	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

6076125033 : MAJOR CLINICAL PHARMACY

KEYWORD: Population pharmacokinetics, Nicotine gum

Kathy Moe San : POPULATION PHARMACOKINETICS OF NICOTINE IN THAI SMOKERS. Advisor: Asst. Prof. Thitima Wattanavijitkul, Ph.D. Co-advisor: Asst. Prof. PAJAREE CHARIYAVILASKUL, Ph.D.

Despite the popularity of nicotine gum in Thailand, population pharmacokinetics of nicotine gum in Thai population has not been investigated yet. This study aimed to develop a population pharmacokinetic model to quantify the effects of genetic and nongenetic factors to nicotine pharmacokinetics after administration of nicotine gum to adult Thai smokers. A population pharmacokinetic analysis was performed using secondary data collected from a previous clinical trial assessing cytochrome P450 2A6 (CYP2A6) genotypes in Thai smokers. Eighteen healthy adult smokers were included in the study. Blood samples were collected at pre-dose, and 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4.5, and 6h after receiving a single dose of 2 mg nicotine gum. Population pharmacokinetics of nicotine was performed using nonlinear mixed effect modeling. One-compartment with 1^{st} order elimination and 1^{st} order absorption with 6-transit compartments best described the data. Enzymatic activity of different CYP2A6 genotypes was a significant covariate on clearance of nicotine. Apparent elimination clearance (CL/F) and apparent volume of distribution (V/F) of nicotine for a typical smoker with normal-function CYP2A6 genotype (or 100% CYP2A6 activity) was 266 L/h and 851 L respectively. Results of 1,000 bootstrapping and visual predictive check showed that model was valid and appropriate. This first report on population pharmacokinetics of nicotine gum in Thai smokers provided the pharmacokinetic model of nicotine and quantified CL/F of nicotine for smokers with different CYP2A6 genotypes.

Field of Study:	Clinical Pharmacy	Student's Signature .....
Academic Year:	2019	Advisor's Signature .....
		Co-advisor's Signature .....

ACKNOWLEDGEMENTS

I would like to express my special appreciation and thanks to my advisor Asst. Prof. Thitima Wattanavichitkul for continuous support of my study, for her patience, and for allowing me to grow as an academic. She consistently allowed this study to be my own work, but steered me in the right direction whenever I needed it. Her guidance helped me in all the time of research and writing of this thesis.

Besides my advisor, I would like to express my sincere gratitude to co-advisor, Asst. Prof. Pajaree Chariyavilaskul for offering an opportunity to do this research and to Dr. Richard Hoglund for his valuable suggestions in my paper. My sincere thanks also go to the rest of my thesis committee, Assoc. Prof. Wanchai Treyaprasert and Dr. Tatta Sriboonruang, for their encouragement and insightful comments.

I would also like to acknowledge Scholarship Program for International Graduate Students in ASEAN countries which was provided by Chulalongkorn University. Their generous contribution has allowed me to focus on my academic pursuits. I would not be able to achieve my Master's degree and to reach my fullest professional potential without their generous support. A special gratitude is extended to Prof. Khin Thet Wai, Prof. Myat Min, Ms. Yee Yee Win, and my teachers and colleagues from Department of Pharmacology, University of Pharmacy (Mandalay), for their enthusiastic help in applying approval from Ministry of Heath and Sports (Myanmar) to join the scholarship program.

Special thanks should be given to my friends, Mrs. Chanika Chuphan and Mr. Vichapat Tharanon, for giving me a hand whenever I needed a favor, for supporting me spiritually during my stay in Thailand, and for all fun we have had together during the last two years. Finally, I must express my very profound gratitude to my parents and to my most beloved one for providing me with spiritual support and continuous encouragement throughout my years of study.

Kathy Moe San

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER I INTRODUCTION

1.1 Background and rationale

Nicotine is the major tobacco alkaloid and mainly responsible for tobacco addiction. Tobacco consumption causes serious health problems such as cancers, cardiovascular diseases and respiratory diseases[1]. Globally, approximately half of life-long smokers died prematurely of tobacco-related diseases[2]. Tobacco-related death accounted for 18% of all death in Thailand[3]. However, tobacco-related diseases are preventable. Quitting smoking has been shown to substantially reduce the risk of mortality[4].

Nicotine replacement therapy (NRT) is the first-line pharmacotherapy for smoking cessation. It relieved nicotine withdrawal symptoms and increased quit rate by 50-60%[5]. Nicotine gum is the first approved NRT and it has a strong evidence of efficacy. A systematic review on 56 clinical trials of nicotine gum reported that nicotine gum was 1.49 times more effective than placebo[5]. Moreover, nicotine gum is the most dispensed NRT for smoking cessation in Thai community pharmacies[6].

Nicotine is a weak base, readily absorbed in basic pH, and widely distributed into body tissues[7-9]. Majority of nicotine (~90% of the dose) is eliminated via hepatic metabolism, which is mediated by Cytochrome PP450 2A6 (CYP2A6) enzyme[7]. Considerable variation in the levels of plasma nicotine concentrations after chewing multiple doses of nicotine gum has been reported in literature[10-12]. A number of pharmacokinetic studies using non-compartmental approach have been conducted with nicotine gum[13-18]. In these studies, oral elimination clearance (CL/F), volume of distribution (V/F), and half-life ($t_{1/2}$) of nicotine after receiving single dose 2-mg nicotine gum were within 54.7-178.6 L/h, 322.1-833.0 L, and 2.0-7.4 h respectively.

CYP2A6, highly polymorphic gene with more than 40 variants, enzyme activity has been shown to influence the rate of nicotine metabolism, which in turn affects the therapeutic efficacy of NRTs[19-24]. Variability in therapeutic response to NRTs was found in groups of different nicotine metabolizers. Population pharmacokinetic analyses are useful to identify sources of variation in a population leading to individualized pharmacologic interventions. Providing individualized pharmacotherapy

might increase the rate of successful smoking cessation and improve the efficacy of NRT.

Despite many non-compartmental analyses of nicotine gum, only one population pharmacokinetic study of nicotine gum has been reported[25]. Marchand performed population pharmacokinetics analysis of nicotine with different kinds of NRTs (nicotine gum and nicotine nasal spray) and tobacco products (cigarettes and tobacco heating system) in UK, USA, and Japan. Among 248 recruited adult smokers, 36 subjects were administered nicotine gum. CYP2A6 enzyme activity and sex was found to have the significant impact on elimination clearance of nicotine and menthol on volume of distribution of nicotine.

However, to the best of our knowledge, population pharmacokinetic analysis has not been conducted yet in Thai population. One clinical trial conducted at King Chulalongkorn Memorial Hospital assessed CYP2A6 allele frequency in 127 Thai smokers. Then, 10 normal metabolizers and 8 slow metabolizers were chosen and provided single dose 2-mg nicotine gum. Differences in PK profiles of nicotine between two CYP2A6 phenotype groups were investigated using non-compartmental approach. This study performed a retrospective population pharmacokinetic analysis on secondary data obtained from that clinical trial. The findings from this study would be valuable to individualized smoking cessation pharmacotherapy.

1.2 Objectives of the study

1. To develop the population pharmacokinetic model of nicotine in healthy adult Thai smokers.

2. To investigate the impact of genetic and non-genetic factors on the pharmacokinetics of nicotine in Thai smokers.

1.3 Scope of the study

This study focused on adult Thai smokers who participated in clinical trial conducted at King Chulalongkorn Memorial Hospital between September 2014 and February 2016 (ethical approval number 085/58).

1.4 Hypothesis of the study

Pharmacokinetics of nicotine could be described by one- or two-compartment model.

Genetic polymorphism in CYP2A6 enzyme and non-genetic factors including body weight, body mass index, monthly alcohol consumption, years of cigarette smoking, number of cigarettes per day and level of nicotine dependence could influence the pharmacokinetics of nicotine.

1.5 Significance of the study

This first report on population pharmacokinetics of nicotine gum in Thai smokers provided the pharmacokinetic model of nicotine and quantified the oral elimination clearance of nicotine for smokers with different CYP2A6 enzyme activities. Pharmacokinetic parameters from this study may be used to provide dosing guidance and personalized therapy for smoking cessation.

CHAPTER II LITERATURE REVIEW

2.1 Tobacco addiction and tobacco cessation pharmacotherapy

2.1.1 Health consequences of smoking

Tobacco smoking is related to serious health problems. A report of surgeon general documented a list of tobacco-related health consequences and diseases (figure 1)[1]. Cigarette smoking is the major cause of lung cancer, chronic pulmonary diseases and cardiovascular diseases. Carcinogens contained in the cigarette smoke lead to the development of cancers. Deposit of smoke particles in the small airway and alveoli causes respiratory diseases. Risk of death from cardiovascular diseases is three times higher in smokers than in non-smokers[1].

In Thailand, death caused by tobacco use accounted for almost one-fifth of the total death[3]. In other words, one in five people died of tobacco-related diseases annually. Cardiovascular death caused by tobacco use was approximately 19% of all cardiovascular deaths each year in Thailand[3]. A large nationwide cohort study reported that current smokers had substantially higher risk of all-cause mortality and smoking cessation substantially reduced the avoidable mortality[26].

2.1.2 Mechanism of nicotine addiction

Nicotine is the principle constituent of tobacco, which is mainly responsible for tobacco addiction. After smoking a cigarette, nicotine is rapidly absorbed by the small airways and alveoli into the pulmonary circulation, and enters the left ventricle and reaches the brain in the short duration[27]. In the brain, nicotine binds to nicotinic cholinergic receptors (nAChRs) and mediates the release of a number of neurotransmitters including dopamine (figure 2).

Dopamine plays an important role in nicotine addiction. Smokers get a feeling of pleasure due to dopamine after cigarette smoking and they want to repeat smoking to experience dopamine-induced pleasurable feeling (positive reinforcing effect of nicotine). On the other hand, insufficient level of nicotine in the blood during nicotine abstinence period produces a feeling of craving and withdrawal symptoms (negative reinforcement of nicotine). The positive and negative reinforcing

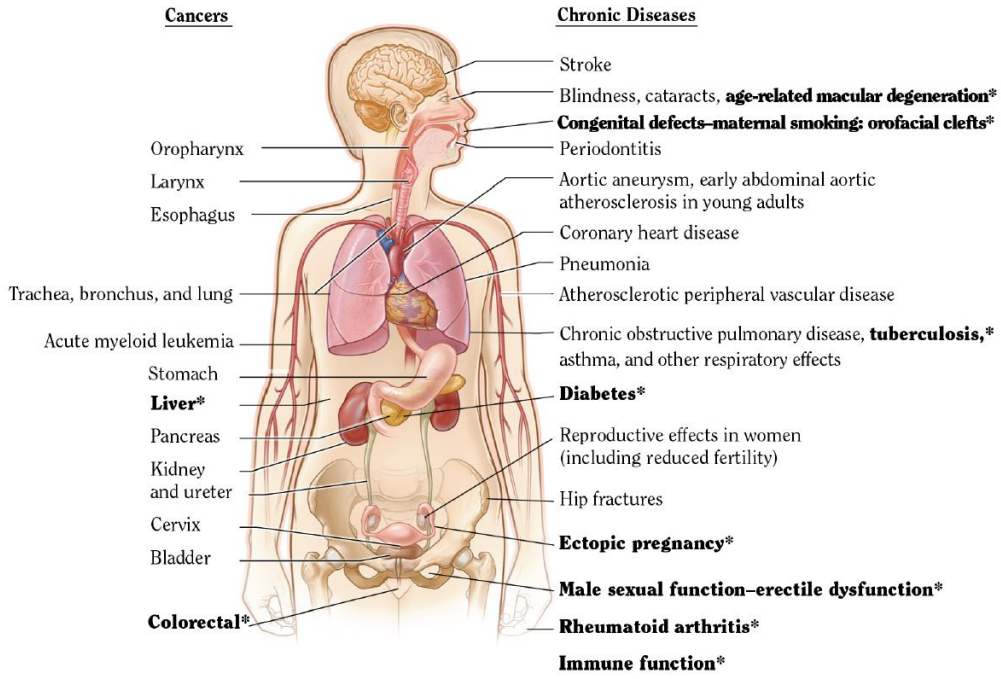

Figure 1 The health consequences causally linked to smoking[1]

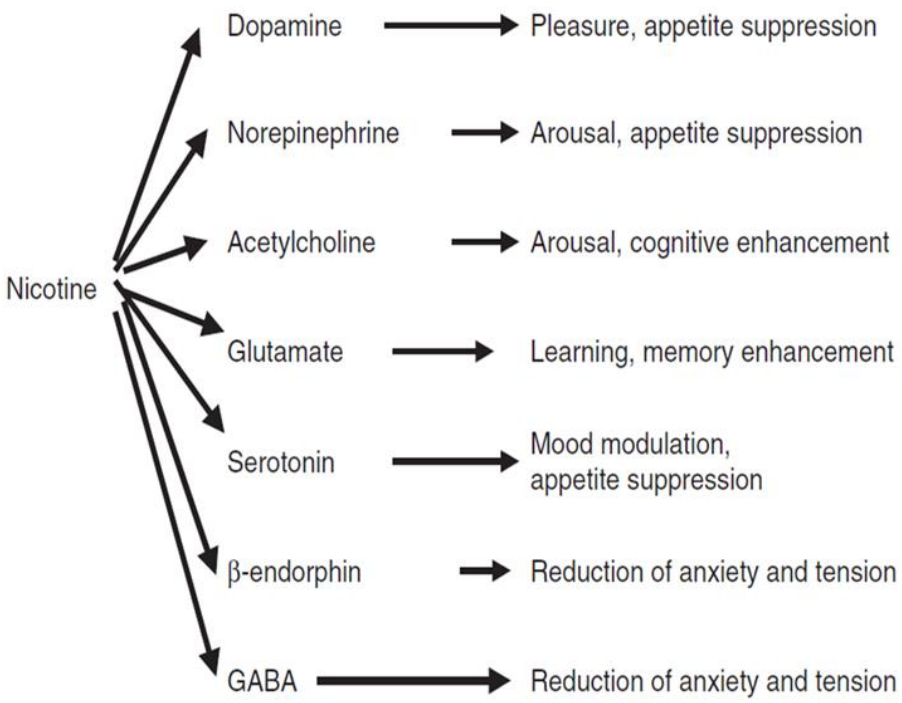

Figure 2 Release of a numbers of neurotransmitters due activation of nicotinic cholinergic receptor by nicotine[27]

effects promote nicotine addiction and causes the continuation of nicotine self-administration.

2.1.3 Nicotine replacement therapy (NRT)

Nicotine replacement therapy (NRT) is the first-line pharmacotherapy for smoking cessation. Different forms of NRT (nicotine gum, lozenge, patch, nasal spray, and inhaler) are available worldwide. Availability of nicotine medications were different between countries. In Thailand, only nicotine gum and nicotine patch are available in Thailand[28] and they could be bought in community pharmacies without prescription. Community pharmacists could provide smoking cessation services and offer an advice to smokers who need help to quit smoking[6].

The major mechanism of action of nicotine medications is that they reduce physiological dependence by delivering nicotine during smoking abstinence[29-31]. NRTs stimulate the nicotinic receptors in the ventral tegmental area of the brain which resulted in the subsequent release of dopamine and reduction in the intensity of craving and nicotine withdrawal symptoms in the smokers who quit smoking. However, all available NRTs could not reproduce the high and rapid delivery of arterial nicotine achieved from cigarette smoking[30]. It took some minutes for oral products (gum and lozenge) and hours for transdermal products (nicotine patch). As a result, the abuse liability of NRTs was considerably low compared to cigarette smoking[32].

All forms of NRTs have a strong evidence of safety and efficacy in over 150 clinical trials[33]. A systematic review reported that NRTs were approximately two times as effective as a placebo[5]. According to the U.S Public Health Service Clinical Practice Guidelines for Treating Tobacco Use and Dependence, smoking cessation pharmacotherapy should be provided to everyone who undergoes a quit attempt except those with special conditions such as pregnancy or breast-feeding, medical contraindications and smoking less than 10 cigarettes daily[34].

Combination therapy of NRTs (long acting transdermal patch plus short acting forms such as gum or lozenges) increased the successful quit by 15-36% compared to a single pharmacotherapy[35]. It has been reported that efficacy of NRTs are not

significantly different between different NRT preparations[5, 35]. A suitable type of nicotine medications should be chosen based on individual's smoking history, previous quit attempts, smoker's medical conditions, and pros and cons of each NRTs[33].

2.1.4 Nicotine chewing gum

Nicotine gum is the most dispensed NRT in Thai community pharmacy[6]. It is available in two strengths: 2 mg and 4 mg. The highly dependent smokers were more likely to quit successfully with high-dose (4-mg) nicotine gum[35]. In the past, level of nicotine dependence was measured by daily cigarette consumption, but some exogenous factors such as increased taxes or increased smoking restrictions may hamper its indicative power[36]. Therefore, an alternative approach for determining nicotine dependence was studied. Time to first cigarette in the morning has been reported as the robust assessment of nicotine dependence and an appropriate basis for nicotine gum dosing algorithm[36]. Smokers who smoke their first cigarette more than 30 minutes after waking up are identified as low dependent and instructed to use 2-mg nicotine gum, whereas those who smoke their first cigarette within 30 minutes are indicated to use 4-mg[37].

The optimal duration of nicotine gum treatment is still unclear. A minimal 12-week dosing algorithm as shown in table 2 is recommended in literature[31, 37]. In the medication package insert, it is suggested that the number of pieces chewed each day should be tapered off after 12 weeks, but the gum use should not be continued beyond 6 months. Hall et al (2009) compared the efficacy of 12-week treatment and extended 40-week treatment of nicotine gum in chronic heavy smokers aged older than 50 years and found no significant differences in efficacy and safety between standard duration and extended duration[38]. However, more studies may be required to conclude the effective duration of gum use.

Table 1 Nicotine replacement therapy and availability in Thailand

Types of Product	Availability in Thailand	Available brands in Thailand
Nicotine gum (2 mg,4 mg)	In pharmacy without a prescription	Nicomild-2, (2mg), Nicorette CoolMint (2mg, 4mg), Nicotinell Mint (2mg)
Nicotine patch (7 mg, 14 mg, 21 mg)	In pharmacy without a prescription	Nicotinell TTS10 (7mg), Nicotinell TTS20 (14mg), Nicotinell TTS30 (21mg)
Nicotine lozenges (2 mg,4 mg)	Not available	Not available
Nicotine Nasal spray (1 mg)	Not available	Not available
Nicotine inhaler (4 mg)	Not available	Not available

Table 2 Indication, dosage and administration of nicotine gum

Dosage	Indication
2 mg gum	For smokers who smoke their first cigarette more than 30 minutes after waking up
4 mg gum	For smokers who smoke their first cigarette within 30 minutes after waking up
Dosage Regimen	
First 6 weeks	One piece every 1-2 hours
Next 3 weeks	One piece every 2-4 hours
Final 3 weeks	One piece every 4-8 hours

2.2 Physicochemical properties of nicotine

Figure 3 Chemical structure of nicotine[9]

Nicotine is the principle tobacco alkaloid. Chemical name is 3-(1-methyl-2-pyrrolidinyl) pyridine. It has a molecular weight of 162.23 g/mol. It is a weak base and has pKa 8.5[9]. It exists as un-ionized form in alkaline medium and ionized form in acidic medium. At physiological pH 7.4, pyrrolidine nitrogen become ionized and pyridine nitrogen remains un-ionized[9]. Therefore, nicotine exists in two states; lipid soluble non-protonated form and water soluble protonated form.

2.3 Pharmacokinetics of nicotine

2.3.1 Absorption

Absorption of nicotine from the mucosal membrane mainly depends on environmental pH[39]. Since nicotine is a weak base, it is highly ionized in acidic media and it cannot be absorbed in acidic pH. In alkaline pH, it is unionized and absorbed readily from the mucosal membrane[8]. Rate and extent of absorption of nicotine mainly depends on type of tobacco product[40]. Absorption from cigarette smoking is the most rapid way in all kinds of nicotine administration. The large surface area in small airways and alveoli and the surrounding pH of 7.4 facilitate the absorption. Nicotine level in the brain reaches the peak within 10-20 seconds after cigarette smoking. In contrast, the absorption from the NRTs is much slower than that from cigarette smoking. Nicotine concentration in the plasma increases gradually after administering NRTs (figure 4)[41].

Absorption of nicotine from nicotine gum is relatively slow and it takes approximately 0.5 to 1 hour to reach the peak level[39]. The surrounding pH of the buccal mucosa has a substantial impact on the absorption of nicotine from nicotine gum (figure 5)[39]. Nicotine absorption from the buccal mucosa increases as pH

increases. Therefore, nicotine gum is buffered to pH 8.5 to facilitate buccal absorption[10, 11] . However, the absorbed amount of nicotine from the gum is less than the actual amount contained in nicotine gum because some amount of nicotine is accidentally swallowed and subjected to hepatic first pass metabolism and some are retained unabsorbed in the gum[12]. Slow increase and low level of peak plasma concentration of nicotine after chewing nicotine gum was assumed as underlying reasons for relatively low success rate of nicotine gum. Bioavailability of nicotine chewing gum was reported as 55-78%[8].

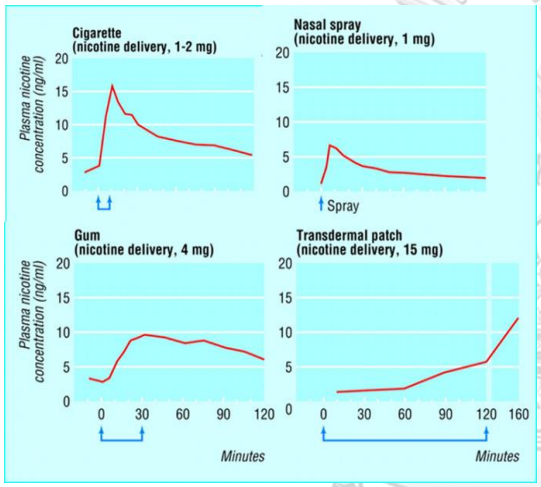

Figure 4 Plasma concentration time profile of nicotine after cigarette smoking and after administering different NRT products[41]
(The blue arrow indicates the period of nicotine delivery.)

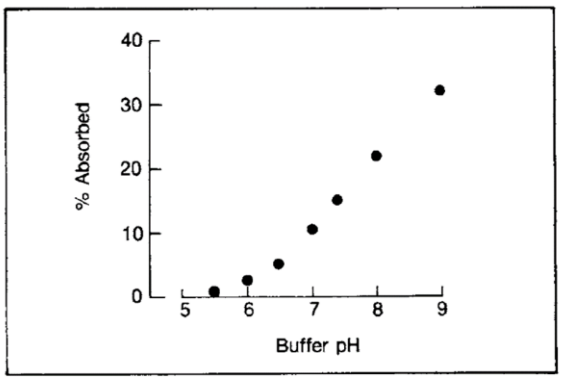

Figure 5 Effect of buffer pH on the buccal absorption of nicotine[39]

2.3.2 Distribution

In blood stream, nicotine is about 69% in ionized form and 31% in unionized form[7]. Nicotine is extensively distributed to the body tissues. Volume of distribution of nicotine after intravenous administration was found ranging from 1 L/kg to 3 L/kg of body weight[39]. Liver, kidneys, lungs and brain are the tissues that have the highest affinity for nicotine. Concentration of nicotine in the skeletal muscle was the same as that in the blood. Plasma protein binding of nicotine is low (approximately 5-20%). Nicotine readily crossed the placenta and was also secreted in milk. Concentration of nicotine in milk was 3 times higher than that in the serum in a nursing mother.

2.3.3 Metabolism

Almost 90% of nicotine was metabolized in the liver. Nicotine was metabolized into six inactive metabolites. Approximately 70-80% of nicotine was metabolized into cotinine. The remaining 10% was metabolized into other metabolic products. Metabolism of nicotine to cotinine undergoes via two-step process. Nicotine is firstly transformed to nicotine-iminium ion by CYP2A6 enzymes. The second step is the conversion of nicotine-iminium ion to cotinine, which is mediated by aldehyde oxidase. Cotinine is also extensively metabolized into different compounds. Only small amount of cotinine is excreted unchanged in urine. The major metabolic product of cotinine is trans-3-hydroxycotinine (3HC). This metabolism is also mediated by CYP2A6 enzyme. Most of 3HC are excreted unchanged in urine and some are excreted as 3HC glucuronide form.

Other metabolic products of nicotine are nornicotine and 4-hydroxy-4-(3-pyridyl)-butanoic acid metabolized by liver cytochrome enzymes, nicotine glucuronide metabolized by uridine diposhphate glucuronosyltransferase (UGT), nicotine N'-oxide metabolized by Flavin-containing monoxoygenase 3 (FMO-3), nicotine isomethonium ion metabolized by amine N'-methyl transferase. The schematic diagram of nicotine metabolism and the percentage of nicotine and its metabolites excreted in urine was shown in figure 6[8].

CYP2A6 gene is highly polymorphic with more than 40 variants. It has the significant impact on rate of metabolism of nicotine. CYP2A6 enzyme was

responsible for approximately 90% of nicotine metabolism to cotinine and the remaining portion 10% was mediated by CYP2B6 enzyme[19]. Although CYP2B6 gene is polymorphic, the relationship between CYP2B6 polymorphism and nicotine metabolism has not been studied yet. Polymorphism in aldehyde oxidase is still unknown.

Genetic variants in UGT and FMO3 were found. Metabolic pathways of nicotine mediated by UGT and FMO3 become prominent in subjects with decreased CYP2A6 enzyme activity. The urinary recovery of nicotine glucuronide, the metabolic product of nicotine mediated by UGT, increased from 3-5% to 40% in smokers who have a complete lack of CYP2A6 enzyme activity[42]. Urinary excretion of nicotine N'-oxide also increased in those with deleted CYP2A6 gene. Therefore, polymorphic impact of UGT and FMO3 on pharmacokinetics of nicotine has been analyzed. Taghavi T. et al reported that UGT2B20, UGT2B17 variants and FMO3 polymorphisms do not have significant impact on nicotine and cotinine pharmacokinetics[42]. According to these literatures, CYP2A6 polymorphism is currently an important source of variation in nicotine metabolism. Polymorphisms in other enzymes play a minor role in pharmacokinetics of nicotine.

2.3.4 Excretion

Very low amount of administered nicotine (approximately 8-10%) was excreted unchanged in urine. Urinary excretion of nicotine involved three processes: glomerular filtration, active tubular secretion and reabsorption. Organic cation transporter (OCT) is a substrate for nicotine and involved in tubular secretion process. Polymorphism in OCT has been reported. OCT nonsynonymous SNP gene variant (OCT2 rs316019) resulted in reduced renal clearance of nicotine and a significant increase in maximum concentration of nicotine and cotinine in the plasma. Reabsorption of nicotine highly depends on urinary pH. In alkaline urine, urinary excretion of nicotine was 1 L/h. In acidic urine, urinary excretion increased to 14-36 L/h depending on the urine flow rate.

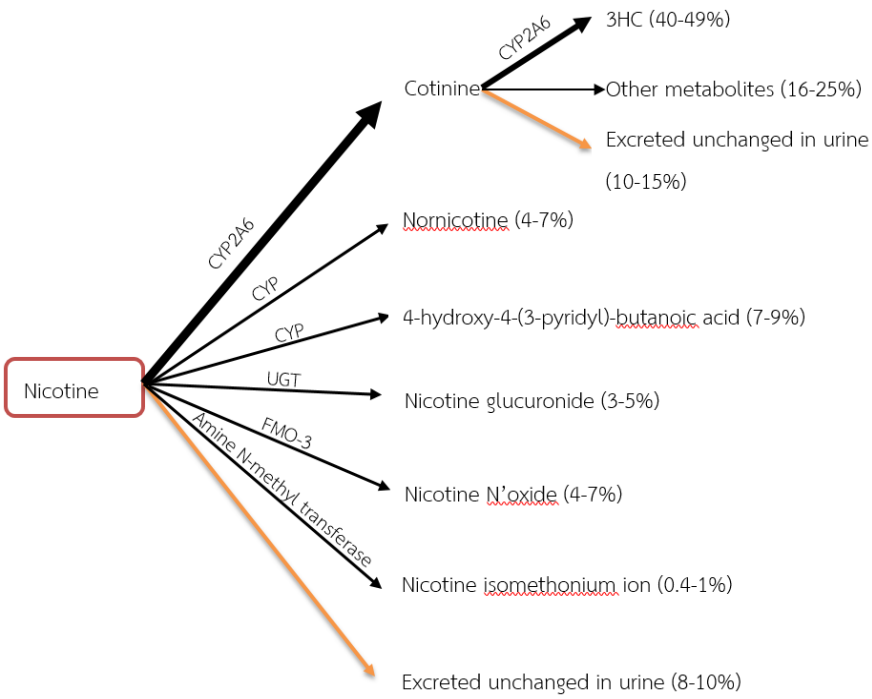

Figure 6 Elimination pathway of nicotine.

(The values in the parenthesis indicated that percentage of nicotine and its metabolites excreted in urine. The value for 3HC combined percent excretion of 3HC and 3HC glucuronide. Adapted from Hukkanen J et al. Pharmacological reviews. 2005 Mar 1;57(1):79-115.)

2.4 Traditional pharmacokinetic studies of 2 mg nicotine gum

Benowitz NL. analyzed the intake of nicotine from nicotine chewing gum[12]. Extraction of nicotine from nicotine gum was incomplete and it accounted for 53% for 2 mg gum. Rate and intensity of chewing influenced the extracted amount. Two-fold inter-individual variability was found in extraction of nicotine from nicotine gum. Systemic intake of nicotine from nicotine gum was less than the actual extracted amount due to accidental swallowing. Approximately three-fold variability was found in the plasma level of nicotine after chewing 2 mg nicotine gum. Absorption of nicotine from buccal mucosa and amount of swallowed nicotine influenced the systemic appearance of nicotine from nicotine gum.

A number of pharmacokinetic studies using non-compartmental analysis have been conducted with nicotine gum[13-18]. These studies compared the pharmacokinetics of new NRT formulations with that of 2-mg nicotine gum in healthy

adult smokers. Study descriptions and pharmacokinetic parameters of single dose Nicorette® 2 mg gum were summarized in table 3.

Table 3 Non-compartmental analyses of single-dose 2-mg nicotine gum

Author (year)	Choi (2003) [a]	Dautzenberg(2007)	Muneesh (2016)	Hansson (2017) [a]	Brossard (2017) [b]		Du (2018)
					Tokyo	Saitama	
Population	USA	French	Indian	Swedish	Japanese		European
N	23	9	43	44	18	18	62
washout period	12 h	24 h	36 h	12 h	At least 24 h		36 h
Blood sampling	14 samples over 12 h	11 samples over 8 h	19 samples over 24 h	19 samples over 12 h	16 samples over 24 h		13 samples over 12 h
Assay method (LLOQ)	LC-MS/MS (1 ng/ml)	GC-MS (1 ng/ml)	LC-MS/MS (0.2 ng/ml)	GC-MS (0.5 ng/ml)	LC-MS/MS (0.2 ng/ml)		LC-MS/MS (0.2 ng/ml)
Chewing time	30 min	30 min	30 min	30 min	35±5 min		30 min
C_{max} (ng/ml)	4.0 ± 1.5	2.9 ± 1.2	7.3 ± 2.1	5.9 ± 1.9	4.8	7.52	3.7 ± 1.3
T_{max} (h)	0.8 ± 0.2	0.8 ± 0.1	0.7 (0.3,3.0)	0.5 [c]	35.4 [d]	45.0 [d]	0.8 (0.5, 1.5)
$AUC_{0\text{-last}}$ (h*ng/ml)	10.7 ± 6.6	10.6 ± 4.4	32.3 ± 11.5	15.1 ± 5.3	14.9	27.9	10.2 ± 3.78
$AUC_{0\text{-inf}}$ (h*ng/ml)	11.3 ± 7.6	13.8 ± 5.6	36.6 ± 13.4	17.1 ± 6.0	16.6	31.1	11.2 ± 4.0
$t_{1/2}$ (h)	2.5 ± 1.2	2.5 ± 1.0	7.4 ± 4.7	2.9 [c]	4.8	3.5	2.0 (1.2, 4.2)
Kel (1/h)	-	-	0.13 ± 0.08	-	-	-	0.3 (0.2, 0.6)
CL/F* (L/h)	177.0	144.9	54.7	117.0	120.3	64.3	178.6
V/F** (L)	638.5	522.8	583.5	455.7	833.0	322.1	523.1

C_{max}: maximum plasma concentration of nicotine; t_{max}: time to maximum plasma concentration of nicotine; $AUC_{0\text{-last}}$: area under plasma concentration-time curve from start of nicotine gum chewing to last sampling time; $AUC_{0\text{-inf}}$: area under plasma concentration-time curve from start of nicotine gum chewing extrapolated to infinity; $t_{1/2}$: plasma elimination half-life; Kel: elimination rate constant.

Values were expressed as Mean ± SD or Medium (minimum, maximum).

[a] Plasma concentrations of nicotine were reported as baseline-adjusted values because of measurable pre-dose concentrations.

[b] Values were expressed as geometric least square mean except T_{max}.

[c] Value was expressed as mean and standard deviation was not reported.

[d] Value was expressed as median and range was not reported.

*CL/F was calculated by Dose/ $AUC_{0\text{-inf}}$.

**V/F was calculated by (CL/F* $t_{1/2}$)/0.693.

2.5 Population pharmacokinetic studies of nicotine

Population pharmacokinetic studies have been conducted with nicotine[25, 43] and these studies were summarized in table 4. Marchand et. al (2017) developed population pharmacokinetic model of nicotine following administration of different preparations of nicotine including nicotine gum. They performed a retrospective population pharmacokinetic analysis on data obtained from four clinical trials. Those trials compared pharmacokinetics and safety of tobacco heating system (regular or mentholated) versus cigarettes (regular or mentholated), nicotine nasal spray and nicotine gum. A total of 248 healthy adult smokers were included in Marchand study. Among them, 38 smokers received 2 mg nicotine gum. It was found that CYP2A6 enzyme activity and sex were significant covariates on clearance of nicotine, menthol increased volume of distribution, and body weight influenced the relative bioavailability of nicotine.

Linakis et. al developed a population pharmacokinetic model of nicotine with transdermal nicotine patch[43]. It was also a retrospective analysis performed on secondary data obtained from previous clinical trial. The previous study investigated the pharmacokinetics of transdermal patch in adult ex-smokers using non-compartmental analysis. Body weight was the only significant covariate on volume of distribution of nicotine in Linakis study.

Table 4 Population pharmacokinetic analyses of nicotine

Author (year)	Subjects	Study design	Blood Sampling	Analysis	Model	Parameters	Covariates
Marchand M et. al (2017)	Healthy Adult Smokers (n=246)	• Combined retrospective analysis of 4 clinical trials • 4 clinical trials: → cross-over studies → conducted in UK, USA, and Japan → compared the PKs of THS with 2-mg nicotine gum (n=36), 1-mg NNS and CC	Pre-dose,10,20, 25,30, 35, 40, 45min, and 1,2, 3,6,9,12,24 h for nicotine gum Pre-dose, 2,4,6,8, 10,15,30,45min, and 1, 2, 4, 6, 9, 12, 24 h for other types of products	LC-MS/MS (LLOQ of 0.2ng/ml) Phoenix® NLME™	Two-compartment with zero-order absorption and first order elimination Pre-dose modelling was included.	CL/F= 24.42 L/h V1/F=70 L V2/F=171 L Q/F= 10.26 L/h C0=0.36 ng/ml	• CYP2A6 activity & sex on CL/F • Menthol on V1/F and Tdur • CYP2A6 activity on C0 • Body Weight on Frel • Type of product on Frel and Tdur
Linakis MW et. al (2017)	Former adult smokers (n=25)	• Retrospective analysis of clinical trial • Clinical trial: → conducted in USA → determined PKs of transdermal nicotine	pre-dose and 0.5, 0.75,1,2,3,4,5,6,8 ,12, and 24h after patch removal	UHPLC-MS/MS (LLOQ of 2.5ng/ml) NONMEM	One-compartment with Weibull-type absorption and first order elimination	CL/F= 90.4 L/h V/F= 104 L	• Body weight on V/F

NNS, nicotine nasal spray; CC, conventional cigarette; THS, tobacco heating system; C0, baseline nicotine concentration; Frel, relative bioavailability; Tdur, duration of zero-order absorption; V1, central volume of distribution, V2= peripheral volume of distribution, CL, clearance; Q, inter-compartmental clearance, LLOQ, lower limit of quantification

2.6 Factors influencing the pharmacokinetics of nicotine

2.6.1 Factors influencing the absorption of nicotine

Type of tobacco products and NRTs influenced the rate and extent of absorption. Absorption of nicotine from inhaled products had the fastest rate of absorption compared with other preparations of nicotine. Rate of absorption of nicotine from nicotine gum was slower than that from inhaled products, but faster than that from nicotine transdermal patch. Body weight has been reported as an influencing factor on relative bioavailability of nicotine[25]. However, explanation for this finding is still unclear.

2.6.2 Factors influencing the distribution of nicotine

Body weight influenced the volume of distribution of nicotine. Increase in body weight increased the volume of distribution of nicotine[43]. Although presence of menthol has been reported to increase the volume of distribution, the reason is still unknown[25].

2.6.3 Factors influencing the elimination of nicotine

a. CYP2A6 Genetic Polymorphism: CYP2A6 gene is highly polymorphic with more than 40 variants[19]. It was reported that some CYP2A6 variants increased the CYP2A6 enzyme activity and some decreased the activity. Different CYP2A6 enzyme activities among individuals caused a wide inter-individual variability in clearance of nicotine. It was found that doubling in CYP2A6 enzyme activity increase the clearance of nicotine by 25%[25].

b. Gender: It was reported that female had faster rate of metabolism and higher clearance of nicotine than male[8]. Reduced clearance in women who did not take estrogen containing oral contraceptive compared with women taking those drugs and no differences in clearance between men and post-menopausal women suggested that estrogen induced CYP2A6 activity. Population pharmacokinetic analysis of nicotine reported that clearance of nicotine was 26% higher in female than male[25].

c. Alcohol: Alcohol abstinence for 7 weeks significantly decreased the rate of nicotine metabolism (approximately 50%) in male alcohol-dependent smokers[44]. It was also reported that reduced intake of alcohol decreased the nicotine metabolite ratio over time[45]. These studies suggested that alcohol induced CYP2A6 activity and alcohol consumption was associated with increase in rate of nicotine metabolism in male smokers.

d. Smoking: Smoking itself inhibited rate of metabolism of nicotine. Reduced clearance of nicotine in smokers compared with non-smokers, 14% increase in clearance of nicotine after 4 days of smoking abstinence[46], and 36% increase in clearance of nicotine after 7 days of smoking abstinence[47] suggested that smoking itself had the inhibiting effect on the metabolism of nicotine.

2.7 Variation in CYP2A6 activity and its effect on smoking cessation outcome

2.7.1 CYP2A6 genetic variation

The functionally significant CYP2A6 genetic variants are the major contributor of inter-individual variability of CYP2A6 activity. CYP2A6*1 is a wild type variant with full or normal function. Whole gene deletion in CYP2A6*4 allele results in fully inactive allele and reduced clearance of nicotine. Complete loss of CYP2A6 enzyme activity was found in individuals who had homozygous CYP2A6*4 allele. Similarly, CYP2A6*10 caused complete lack of enzyme activity toward nicotine as *4. Most of the other variants resulted in decreased activity of CYP2A6 enzymes.

Distribution of CYP2A6 variant alleles are different in different ethnic groups. Frequency distribution of CYP2A6 genotypes in Asian population including Thai and their impact on enzymatic activity are summarized in table 5. CYP2A6 genotypes data for Thai population are obtained from Mahavorasirikul, W. et al. 2009 [48] and the original clinical trial of the current study (unpublished data). More than half of Thai individuals (almost 53%) have normal-function CYP2A6 genotypes (CYP2A6*1/*1). Chinese and Indian populations tend to have higher frequencies of normal-function CYP2A6 genotypes compared to Thai and Japanese population. Higher prevalence of

individuals with loss-of-function and decreased CYP2A6 genotypes was found in Japanese population compared to other populations.

Table 5 Frequency distribution of CYP2A6 genotypes in Asian population

CYP2A6 genotype	Enzyme activity of CYP2A6 genetic variant	Frequency of CYP2A6 genotypes (%)						
		Thai [a,b]		Japanese [c,d]		Chinese [e,f]		Indian [g]
		n=127 [a]	n=194 [b]	n=279 [c]	n=750 [d]	n=279 [e]	n=102 [f]	n=700
*1/*1	N/N	59.9	45.4	16.1	68.1	86.7	83.3	86.3
*1/*4	N/n	13.4	14.4	19.7	28.7	10.4	16.7	12.3
*4/*4	n/n	4.0	0.5	4.3	3.2	2.9	-	1.4
*4/*9	n/D	2.4	3.1	7.9	-	-	-	-
*1/*7	N/D	-	10.3	10.0	-	-	-	-
*1/*9	N/D	14.2	18.6	11.1	-	-	-	-
*1/*10	N/n	-	3.6	4.0	-	-	-	-
*7/*9	D/D	-	2.1	7.9	-	-	-	-
*9/*9	D/D	6.3	-	4.3	-	-	-	-
Others	-	-	2.1	14.7	-	-	-	-

N/N, normal/normal; N/n, normal/none; n/n, none/none; N/D, normal/decreased; D/D, decreased/decreased;
a. original clinical trial of the current study (unpublished data), b. Mahavorasirikul et al. 2009[48], c. Kumondai et al. 2016[49], d. Ariyoshi et al. 2002[50], e. Wang et al. 2017[51], f. Zhao et al. 2017[52], g. Ruwali et al. 2009[53]

2.7.2 Phenotypic measure of CYP2A6 activity

Nicotine metabolite ratio (NMR), the ratio of trans-3-hydroxycotinine and cotinine (3HC/Cotinine), has been validated as a phenotypic measure of CYP2A6 activity[54]. CYP2A6 is 90% responsible to metabolize nicotine to cotinine, but 100% responsible to metabolize cotinine to 3HC. 3HC was not generated in subjects with non-function CYP2A6 genotype (CYP2A6*4/*4) after administration of nicotine. The long half-life of cotinine (approximately 11 h) and formation dependency of 3HC supported that 3HC/cotinine ratio is a specific and reliable metabolic marker of CYP2A6 activity.

An alternative approach to phenotyping CYP2A6 activity is the activity score (AS) assignment system[55, 56]. The scoring system attempts to translate genotype information into phenotype prediction in a simplified way. Briefly, each allele is given a value reflecting full or normal function (value = 1), decreased function (value = 0.5), and no function (value = 0). The summation of the values for both alleles represents the activity score of each genotype. For example, CYP2A6*1/*1 and CYP2A6*4/*4 has AS of 2 and AS of 0, respectively. Although this system was first developed for phenotyping CYP2D6 activity by Gaedigk et al (2008)[56], it has been adopted in phenotypic prediction of other enzymes including CYP2A6[49, 57]. Due to its simplified approach and clinical utility, the AS system has been widely utilized in many literatures and accepted in clinical settings to provide genotype-based dosing guidance[58].

2.7.3 Variability in treatment outcomes of nicotine replacement therapy

Variability in therapeutic response to nicotine replacement therapy has been found in literature. Four studies have shown that slow metabolizers, defined by the slowest (4th) quartile of 3HC/cotinine ratio, tend to have a significantly higher cessation rates than normal metabolizers, defined by the upper three quartiles of 3HC/cotinine ratio, when treated with nicotine patch or nicotine gum[20, 22-24]. On the other hand, the opposite trend was found in Chen study, with lower relapse rate in normal metabolizers when treated with nicotine patch, nicotine lozenges or combination therapy of patch and lozenges[21]. Differences in study designs including recruited subjects, metabolizer segmenting, provided pharmacological treatment and behavioral counseling, and outcome assessment between studies might contribute to inconsistent results. The previous 4 studies compared efficacy of NRTs across groups of metabolizers without inclusion of placebo arm, whereas Chen included placebo arm and compared efficacy of NRT to placebo in different metabolizer groups. Despite discrepancies between study findings, the influence of CYP2A6 activity on the effectiveness of nicotine replacement therapy is significant in all studies. Consequently, those studies suggested a personalized medicine for smoking cessations based on genetic determination.

CHAPTER III METHODOLOGY

3.1 Study design

A retrospective population pharmacokinetic analysis was performed on anonymous secondary data collected from a previous clinical trial conducted at King Chulalongkorn Memorial Hospital (Bangkok, Thailand) in 2014-2016.

3.2 Original study explored

Original study assessed CYP2A6 genotypes in 127 Thai subjects who attended their medical check-up at King Chulalongkorn Memorial Hospital. Among them, 10 normal metabolizers and 8 slow metabolizers were enrolled in the pharmacokinetic study. The objective of the study was to investigate the pharmacokinetic profile of nicotine following single dose 2 mg nicotine gum administration using non-compartmental approach. Subjects who smoked every day in 5 months prior to the study with an average of approximately 10 cigarettes per day were included in the study. Subjects who were consuming food or drugs that were CYP2A6 inducer or inhibitors, subjects who had a history of chewing disorders or abnormalities in jaw joints, subject with liver or kidney insufficiencies and pregnant and breast-feeding women were excluded in the pharmacokinetic study. The clinical trial was approved by institutional review board of the Faulty of Medicine, Chulalongkorn University, Bangkok, Thailand (Approval number 085/58).

3.2.1 Nicotine gum administration and pharmacokinetic sampling

Subjects were directed to abstain from any form of nicotine for 12 hours prior to the study and to refrain from any sour juice for 30 minutes before the study. Nicotine gum 2mg (Nicotinell®, manufactured by Fertin Pharma A/S, Vejie, Denmark) was administered orally and chewed as instructed for 30 minutes. Blood samples were collected before administration of nicotine gum (pre-dose) and 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4.5 and 6 hours after the start of nicotine administration.

3.2.2 Determination of nicotine concentrations in the plasma

Nicotine concentrations in the plasma were determined by a validated LC-MS/MS using liquid-liquid extraction with the use of similar method to Ghosheh, et

al[59]. The calibration curve ranged from the lower limit of quantification (LLOQ) of 0.25 ng/ml to 50.0 ng/ml. The inter-day and intra-day accuracy was within 93-103% and the coefficient of variation did not exceed 8%.

3.3 Study patients

3.3.1 Population of the study

Population of the study was healthy adult Thai smokers.

3.3.2 Sample population

Participants in the traditional pharmacokinetic study of the previous clinical trial were included in the current study.

3.3.3 Sample size

Eighteen adult Thai male smokers with different CYP2A6 genotypes were included in the study. A total of 172 plasma concentrations of nicotine was used in the population pharmacokinetic analysis.

3.4 Data analysis

3.4.1 Demographic data analysis

Demographic data of the patients was analyzed by Microsoft Excel 2016 and SPSS version 22.0 (SPSS CO., Ltd, Bangkok Thailand). Categorical data was presented as frequency and percent. Continuous data was presented as mean ± SD or median (range).

3.4.2 Population pharmacokinetic modelling

The population pharmacokinetic model of nicotine was developed using non-linear mixed effect modelling approach as implemented in the NONMEM software, version 7.3.0 (ICON Development Solutions, Ellicott city, MD, USA)[60]. The NONMEM runs were performed by PDx-Pop version 5.2.1 (ICON Development Solutions, Ellicott city, MD, USA). Data checkout and model diagnostics were performed via Xpose program (version 4)[61]. The first-order conditional estimation method with interaction (FOCE-I) was used throughout model building process. Three steps were involved in

developing the population pharmacokinetic model; base model development, covariate model development and model evaluation.

Base model development

Base model consisted of two components; structural model and statistical model. A base structural model is a model that describes the general and typical PK characteristics of a drug including distribution compartments, absorption and elimination profile of a drug without consideration of covariates. After exploring the informational content of the data, one- or two- compartment linear models were examined for plasma concentration time profile of nicotine. Since the irregular absorption profile was found in observed plasma concentration-time profile of nicotine, different kinds of absorption model including typical (first- and zero- order absorption) and atypical models (two parallel first-order absorption[62], mixed zero- and first-order absorption[62], first-order absorption with fixed transit compartments[63] and Weibull-type absorption model[43, 64]) were tested. In order to describe the complex nature of absorption profile of nicotine, NONMEM code was written manually by using ADVAN 6.

Statistical model describes the inter-individual variability (IIV) in the PK parameter estimates of a drug among individuals in a population and the unexplained residual variability (RUV) of a drug between predictions and observations. The IIV in the PK parameters was assumed to be log-normally distributed, therefore exponential function was used to model the IIV as described in the following equation:

$$P_i = TVP \times e^{\eta_i}$$

where P_i is the individual model-predicted PK parameter, TVP is the population mean or typical value of that PK parameter, and η_i is the value of the deviation from the typical value for the i^{th} subject.

Four kinds of RUV models were investigated to describe the unexplained variability between observed and predicted data.

Additive model: Y = F + ERR (1)

Proportional model: Y = F * ERR (1)

Combined additive and proportional model: Y = F + F * ERR (1) + ERR (2)

Exponential model: Y = F * EXP (ERR (1)),

where Y is the observed concentration, F is the predicted concentration, and ERR is the value of difference between observed and predicted concentrations.

In addition to structural and statistical model, a component describing the disposition of pre-dose concentration was included in the base model. Despite 12 h nicotine abstinence period, concentrations at time zero were measurable. Therefore, those pre-dose concentrations were modelled by a decreasing mono-exponential term as described in previous literature[25, 65].

The most appropriate base model was chosen by examining the basic goodness-of-fit plots including observed versus individual prediction, observed versus population predictions, and conditional weighted residuals versus time/population predictions, precision of parameter estimates, objective function value (OFV), and akaike information criterion (AIC).

Covariate model development

Covariate model was developed by using a stepwise approach. In forward addition step, a decrease in objective function value (OFV) of >3.84 ($\chi^2_{0.05}$) was considered significant. In backward elimination step, an increase in OFV of >10.83 ($\chi^2_{0.001}$) was considered to retain the covariate in the model.

Depending on the relationship between pharmacokinetic parameters and continuous covariates, linear, power, and exponential models were analyzed as described in the following equations.

Linear covariate model: $P_i = \theta 1 + \theta 2 * (COV - COV_{median})$

Power covariate model: $P_i = \theta 1 * (COV/COV_{median})^{\theta 2}$

Exponential covariate model: $P_i = \theta 1 * e^{(\theta 2 * (COV - COVmedian))}$,

where P_i is individual value of PK parameter, $\theta 1$ is the typical value of population PK parameter, $\theta 2$ is the estimate that reflects the change in PK parameter per unit change in covariate value, COV is the value of continuous covariate, and COV_{median} is the median value of continuous covariate.

The relationship between PK parameters and categorical covariate was modelled by using additive shift function as follow.

For categorical covariate with only two possible levels (e.g., 2 groups of phenotypes),

$$P_i = \theta 1 + \theta 2 * COV,$$

where $\theta 1$ is typical value of PK parameter for one attribute of a dichotomous covariate (e.g., normal metabolizer), $\theta 2$ is the increment or decrement in the PK parameter associated with COV, another attribute of the dichotomous covariate (e.g., slow metabolizer).

For categorical covariates with more than two levels (e.g., 3 levels of nicotine dependence: low, moderate and high),

$$P_i = \theta 1 + \theta 2 * COV1 + \theta 3 * COV2$$

where $\theta 1$ is typical value of PK parameter for reference population (e.g., subject with low level of nicotine dependence), $\theta 2$ is the increment or decrement in PK parameter associated with COV1, an indicator variable of a particular covariate (e.g., subject with moderate level of nicotine dependence), compared to reference population and $\theta 3$ is the increment or decrement in PK parameter associated with COV2, another indicator variable (e.g., subject with high level of nicotine dependence).

Monthly alcohol consumption, Fagerstrom Test for Nicotine Dependence (FTND) score and number of cigarettes per day were studied as categorical covariates on clearance of nicotine. The impact of CYP2A6 genetic polymorphism on the clearance of nicotine were tested in two different ways; two groups of CYP2A6 phenotype (normal metabolizers and slow metabolizers) as a categorical covariate and activity of CYP2A6 genotype (%) as a continuous covariate which is defined as the following equation.

Activity of CYP2A6 genotype (%) = (AS of genotype/AS of full-function genotype) *100 ..... (1)

The activity score (AS) was assigned to each CYP2A6 genotype based on known enzymatic activity of CYP2A6 variants as described in previous literature[49]. We transformed AS of each genotype into percentage value in order to facilitate model run. The impact of body weight and body mass index on volume of distribution of nicotine were studied. Moreover, the effect of CYP2A6 enzyme activity (%) and

monthly alcohol consumption on baseline concentration of nicotine were explored as well.

Model evaluation

Final model was evaluated by using bootstrap analysis and prediction-corrected visual predictive check methods[66, 67]. Parameter precision was evaluated via bootstrap techniques using 1000 randomly sampled datasets. Predictive performance of the model was evaluated with visual predictive checks. The magnitude of eta shrinkage (shrinkage in empirical Bayes estimates) and epsilon shrinkage (shrinkage in individual predictions) was investigated to evaluate the informative value of individual data[67]. Overview of population pharmacokinetic modelling was shown in figure 7.

3.5 Ethical considerations

The research was conducted according to basic ethic principles for research involving human subjects.

1. Respect for person: Everyone should have an autonomy to be involved in the research. Therefore, adequate information was provided to make their own judgment and involvement in research is purely voluntary. Necessary information was provided directly to person who is capable of self-determination and to the care-givers for those who lose the capacity of self-determination.

2. Beneficence: Research investigations should be conducted not to harm the volunteers and to maximize the possible benefits.

3. Justice: The subjects should be selected in well-considered fair procedures in order to ensure the fair distribution of benefits and risk to research candidates.

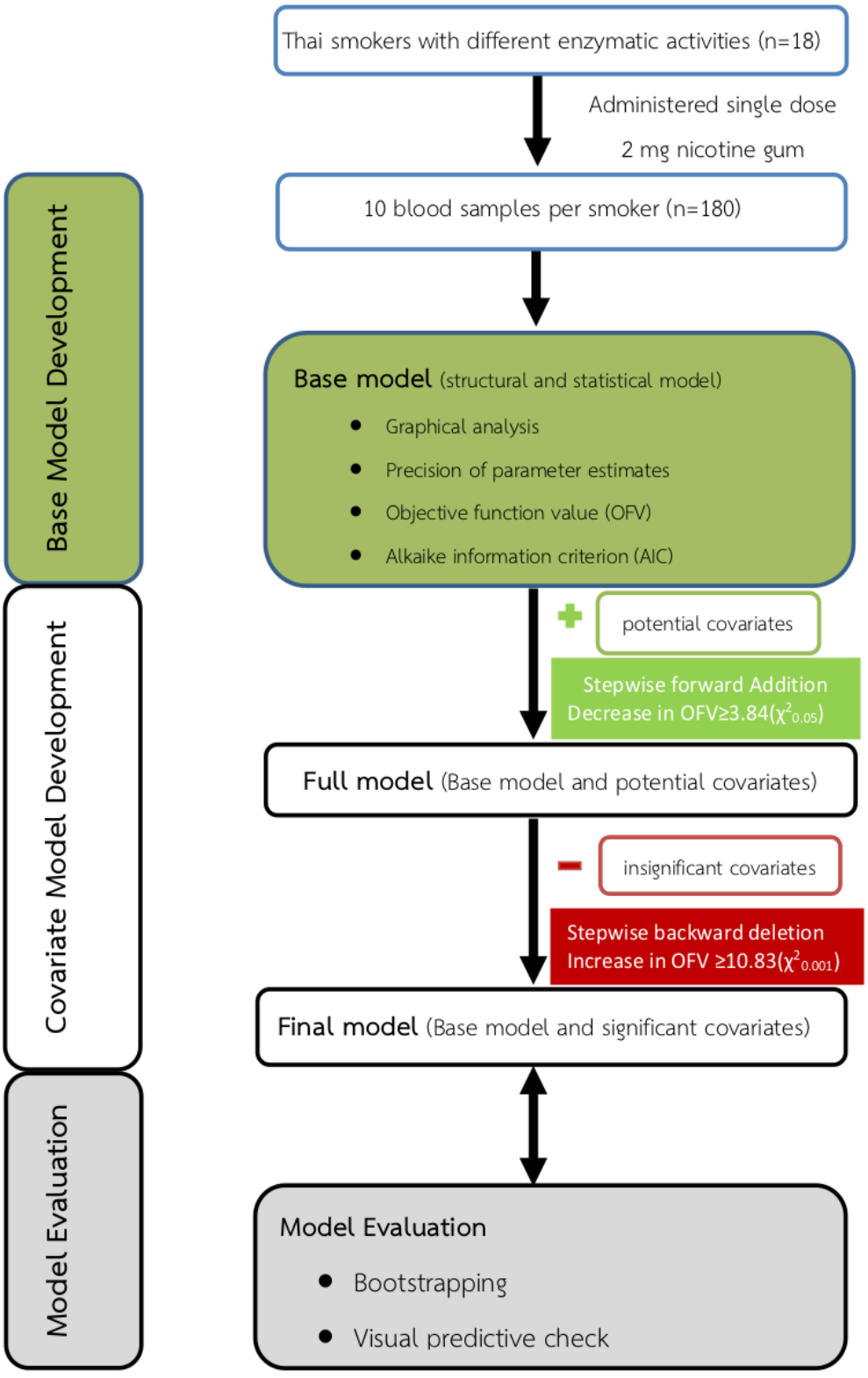

Figure 7 Overview of population pharmacokinetic modelling

CHAPTER IV RESULTS

4.1 Demographic data

Patient characteristics were presented in table 6. More than half of the smokers had low level of nicotine dependence (FTND≤3) and smoked ≤10 cigarettes per day. The median age was 33 years. The median body weight and the median body mass index were 70.5 kg and 24.6 kg/m^2 respectively. Six different CYP2A6 genotypes were included in the study and the percentage of enzymatic activity of CYP2A6 genotypes was shown in table 7. CYP2A6 genotype *1/*1 is wild type and has normal enzyme activity. The remaining CYP2A6 genotypes have decreased enzyme activity and therefore subjects with decreased enzymatic activity were defined as slow metabolizer. Enzymatic activity of CYP2A6 genotypes ranged from 0% to 100%. Five subjects were known to have consumed alcohol on a monthly basis.

4.2 Base model development

After exclusion of 8 concentrations below the limit of quantification (~4%), 172 concentrations were available to develop population pharmacokinetic model. The plasma concentration time profile of nicotine was shown in figure 8. Baseline concentrations were measurable in 11 subjects. Models containing a component describing nicotine disposition of pre-dose concentration significantly improved the model fit more than models without pre-dose modelling (table 8).

Two compartment disposition model did not converge successfully and was not used. One compartment model with 1st order elimination adequately described the observed data. First order absorption with 6 transit compartments was superior than all other investigated absorption models (ΔOFV= -35.9 and -10.3 in compared with 1st order absorption and zero-order absorption, respectively). Addition of more transit compartments did not improve the fit. Weibull, serial first-order, and mixed zero- and first-order absorption models did not converge successfully and were not used.

Due to high variability during absorption phase, the first-order absorption rate constant could not be appropriately estimated and was fixed to 2.9 h^{-1}, based on model fit. The robustness of the fixed value was verified using a sensitivity analysis

by varying the value from 1.8 to 4.4 h^{-1}; the variance model parameter values indicated the chosen value of 2.9 to be appropriate. A proportional error model was chosen to describe the residual variability based on suitability or plausibility of parameter estimates. The results of base model including parameter estimates was shown in table 9. Basic goodness-of-fit plots of base model was presented in figure 9.

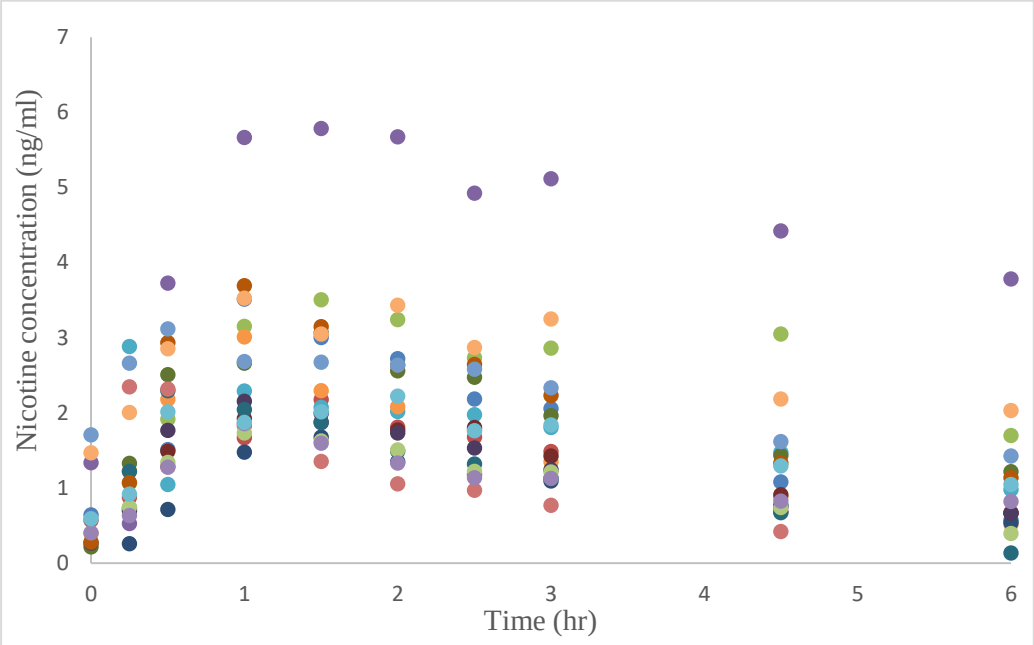

Figure 8 Plasma concentration time profile of single dose 2-mg of nicotine gum

Table 6 Patient characteristics

Characteristics	**Value**
	Median (Range)
Age (year)	33.0 (26.0-58.0)
Actual body weight (kg)	70.5 (57.0-112.0)
Adjusted body weight (kg)	68.5 (58.1 – 85.5)
Ideal body weight (kg)	65.5 (56.9 – 75.0)
Body Mass Index (kg/m^2)	24.6 (19.7-37.9)
CYP2A6 activity (%)	100 (0-100)
Years of smoking (year)	15.5 (8.0-34.0)
	No. (%) of patients
Obesity (BMI>25)	
Yes	9 (50%)
No	9 (50%)
Monthly alcohol consumption	
Yes	5 (27.8%)
No	13 (72.2%)
Number of cigarettes per day	
≤10	16 (88.9%)
11-20	2 (11.1%)
FTND score	
Very low(0-2)	9 (50.0%)
Low (3-4)	7 (38.9%)
Medium (5)	-
High (6-7)	2 (11.1%)
Very high (8-10)	-

Table 7 Enzymatic activity of CYP2A6 genotypes according to Activity Score system

CYP2A6 Phenotype	CYP2A6 Genotype	Activity score	Activity percent	No. (%) of subjects
Normal metabolizer	*1/*1	2.0	100%	10 (55.6%)
	*1/*9	1.5	75%	1 (5.6%)
	*1/*4	1.0	50%	2 (11.1%)
Slow metabolizer	*9/*9	1.0	50%	3 (16.7%)
	*4/*9	0.5	25%	1 (5.6%)
	*4/*4	0	0%	1 (5.6%)

Table 8 OFV and AIC of linear one-compartment with different absorption models and different RUV models with pre-dose modelling and without pre-dose modelling

Absorption model	RUV model	Without pre-dose modelling		With pre-dose modelling		ΔOFV
		OFV	AIC	OFV	AIC	
Zero-order absorption	Add	-41.274	-27.274	-114.883	-96.883	-73.609
	Prop	8.357	22.357	-125.159	-107.159	-133.516
	Comb	NE[a]		-133.663	-113.663	-
	Expo	NE[a]		-125.159	-107.159	-
First-order absorption	Add	-47.666	-33.666	-93.998	-79.948	-46.332
	Prop	8.731	22.732	-108.013	-94.013	-116.744
	Comb	NE[b]		-155.395[#]	-99.395	-
	Expo	-56.089	-42.089	-108.013	-94.013	-51.924
First-order absorption with 6 transits	Add	-64.096	-46.096	-142.381	-124.381	-78.285
	Prop	6.139	24.139	***-143.931***	***-125.931***	***-150.070***
	Comb	NE[b]		-154.252[#]	-134.498	-
	Expo	-65.233	47.233	-143.931	-125.931	-78.698

OFV, objective function value; AIC, Akaike Information Criterion; RUV, residual unexplained variability; Add, additive error; Prop, proportional error; Comb, combined additive and proportional error; Expo, exponential error;

NE[a], model did not converge successfully. NE[b], covariance step was not successful.

ΔOFV, differences in OFV value between models without pre-dose modelling and models with pre-dose modelling. [#]95% confidence interval of additive error (sigma parameter estimate) include zero although model converged successfully, indicating that that parameter is not necessary in the model.

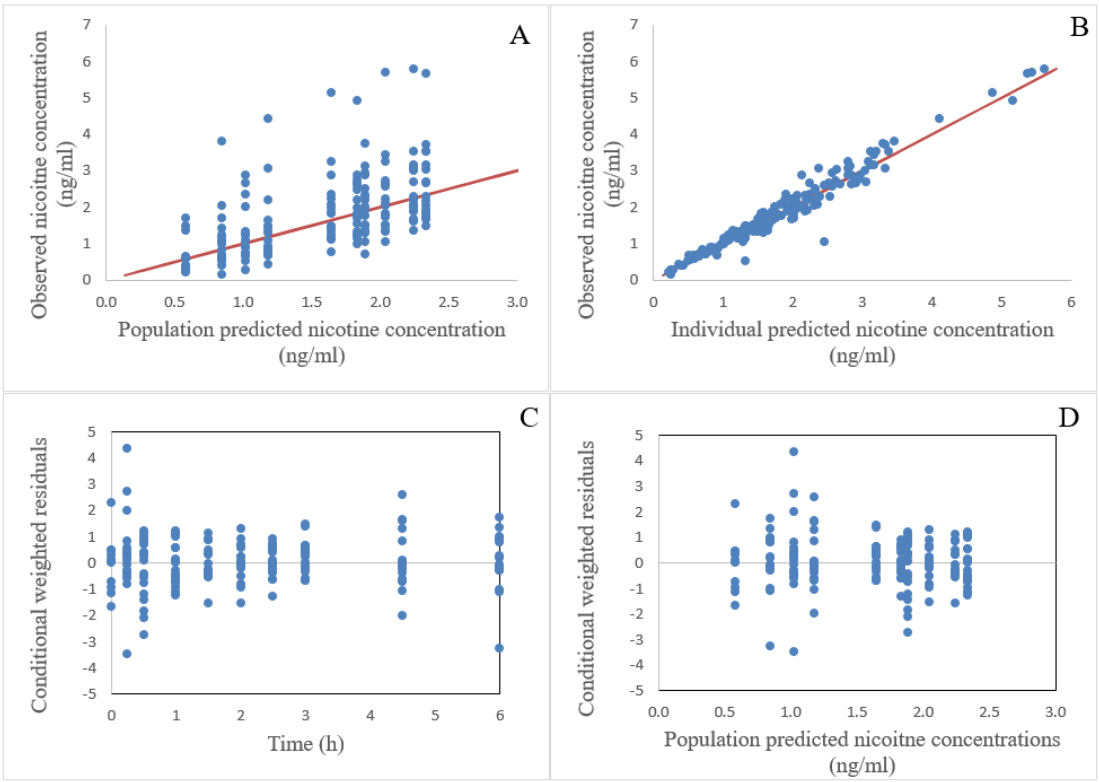

Figure 9 Basic goodness-of-fit plots of base model

A. observed vs. population predicted nicotine concentrations, B. observed vs. individual predicted nicotine concentrations, C. conditional weighted residuals vs. time, D. conditional weighted residuals vs. population predicted nicotine concentrations

Table 9 Population PK parameter estimates of base model

Parameter (unit)	Population estimate [%RSE]	CV% of IIV or RUV [%RSE]	Shrinkage (%)
CL/F (L/h)	190 [14.9]	64.9 [43.5]	3.2
V/F (L)	856 [10.3]	40.4 [32.8]	4.8
K_a (h^{-1})	2.90 *fix*	-	-
MTT (h)	0.12 [15.8]	51.6 [29.2]	12.9
C0 (ng/ml)	0.58 [19.9]	72.5 [23.0]	3.2
RUV	0.02 [24.7]	14.7 [24.7]	18.7

RSE, relative standard error; CV, coefficient of variation; IIV, inter-individual variability; RUV, residual unexplained variability; CL/F, apparent elimination clearance; V/F, apparent volume of distribution; K_a, first-order absorption rate constant; MTT, mean transit time; C0, baseline concentration

4.3 Covariate model development and final model

The relationship between studied covariates and PK parameters were shown in figure 10-13. The results of forward addition step 1 were presented in table 10. Two covariates were significant in forward addition step 1; enzymatic activity of CYP2A6 genotypes (%) on CL/F and monthly alcohol consumption on baseline concentrations of nicotine.

Regarding apparent elimination clearance (CL/F), the impact of CYP2A6 polymorphism on CL/F of nicotine was tested in two different ways; percentage of CYP2A6 enzyme activity as continuous covariate and two groups of CYP2A6 phenotypes (normal and slow metabolizers) as categorical covariate. Addition of CYP2A6 enzyme activity (%) as linear model provided the better model fit than power and exponential models and addition of groups of CYP2A6 phenotype as a categorical covariate (table 10). Therefore, the linear model of CYP2A6 enzyme activity (%) was chosen as a significant covariate to explain the inter-individual variability of CL/F.

Regarding baseline concentration of nicotine, monthly alcohol consumption influenced the baseline concentrations of nicotine significantly (ΔOFV= -5.176 ($\chi^2_{0.05}$)) and reduced the coefficient of variation of the inter-individual variability in baseline concentrations of nicotine from 72% to 61.6%. Other covariates on pharmacokinetic parameters of nicotine were not significant.

The relationship between CYP2A6 enzyme activity (%) and CL/F of nicotine provided the larger decrease in OFV than the relationship between monthly alcohol consumption and baseline concentrations of nicotine. Therefore, the relationship between CYP2A6 enzyme activity (%) and CL/F was added first to the base model. Then, the impact of monthly alcohol consumption on baseline concentration of nicotine was investigated by including it into base model with the relationship between CYP2A6 enzyme activity and CL/F. The results of forward addition step 2 were shown in table 11. The full model consisted of two significant covariates and was described as following equations 2 and 3.

$$CL/F\ (L/h) = 264 + 2.31*(CYP2A6-100) \quad \ldots\ldots\ldots\ (2),$$

$$C0\ (ng/ml) = 0.73 - 0.46*ALCOHOL \quad \ldots\ldots\ldots\ldots\ (3);$$

where CYP2A6 is the enzymatic activity of CYP2A6 genotypes (%) and C0 is baseline concentrations of nicotine. If the subject drinks alcohol in a monthly basis, C0 will decrease by -0.46 ng/ml.

After forward addition step, backward elimination step was performed by removing each covariate from the full model. The influence of CYP2A6 enzyme activity (%) on CL/F remained significant at p-value<0.001 level, but the relationship between monthly alcohol consumption and baseline concentrations of nicotine was insignificant in backward elimination step (table 12). Therefore, the impact of CYP2A6 enzyme activity on CL/F of nicotine was included in the final model, which was described in equation 4.

$$CL/F\ (L/h) = 266 + 2.34*(CYP2A6-100) \quad \ldots\ldots\ldots\ (4)$$

The population pharmacokinetic parameters of final model ware shown in table 13. The final model reduced coefficient of variation of IIV in CL/F from 64.9% to 38.5% compared with base model. According to final model described in equation 4, CL/F of nicotine for a typical person with normal-function CYP2A6 genotype (or 100% CYP2A6 activity) was 266 L/h. If the CYP2A6 activity decreased 25%, the CL/F decreased by 58.5 L/h (or 22%). CL/F would be 32 L/h (or 12% of the typical value) in a subject with non-function CYP2A6 genotype (or 0% CYP2A6 activity).

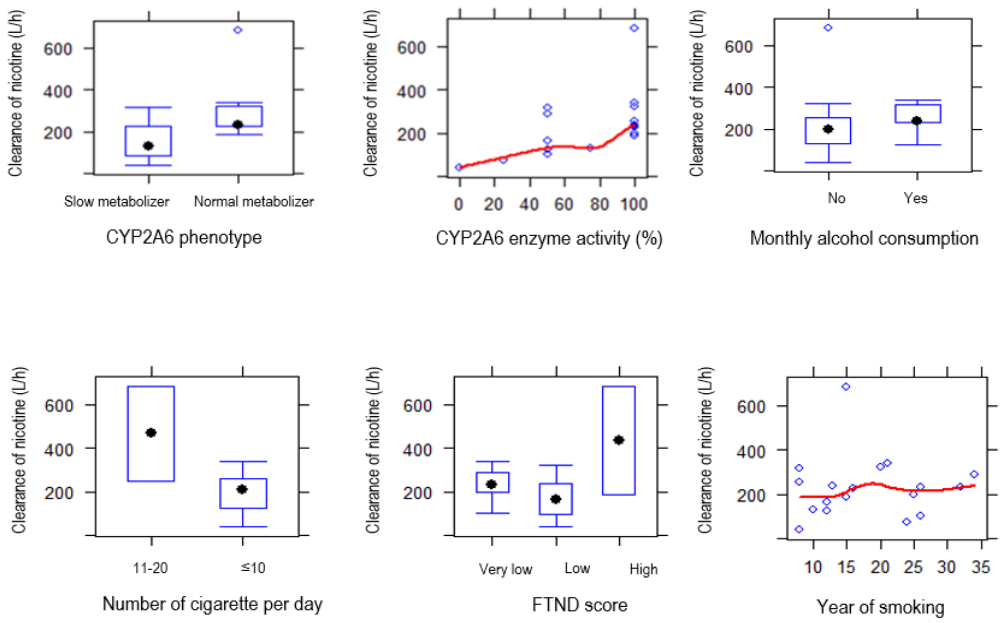

Figure 10 Plots of the relationship between covariate values and oral clearance of nicotine

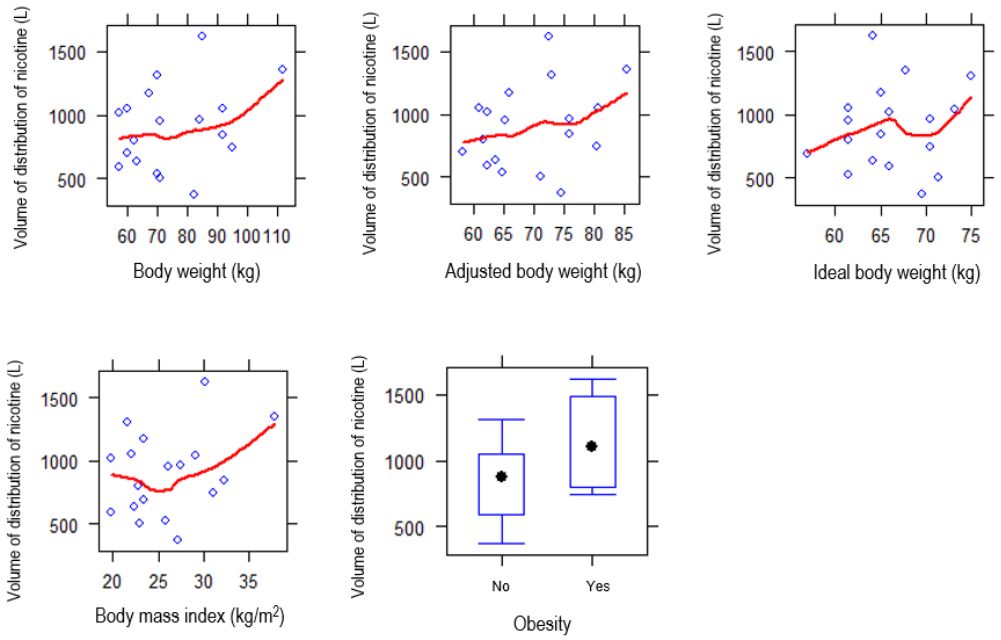

Figure 11 Plots of the relationship between covariate values and volume of distribution of nicotine

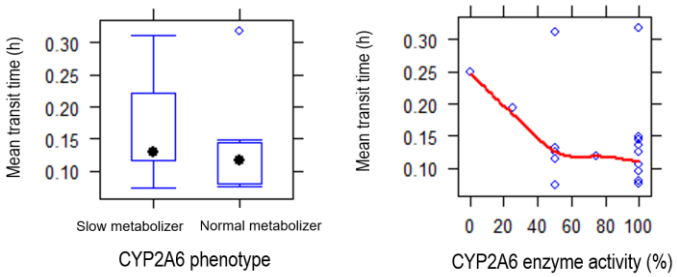

Figure 12 Plots of the relationship between covariate values and mean transit time of nicotine

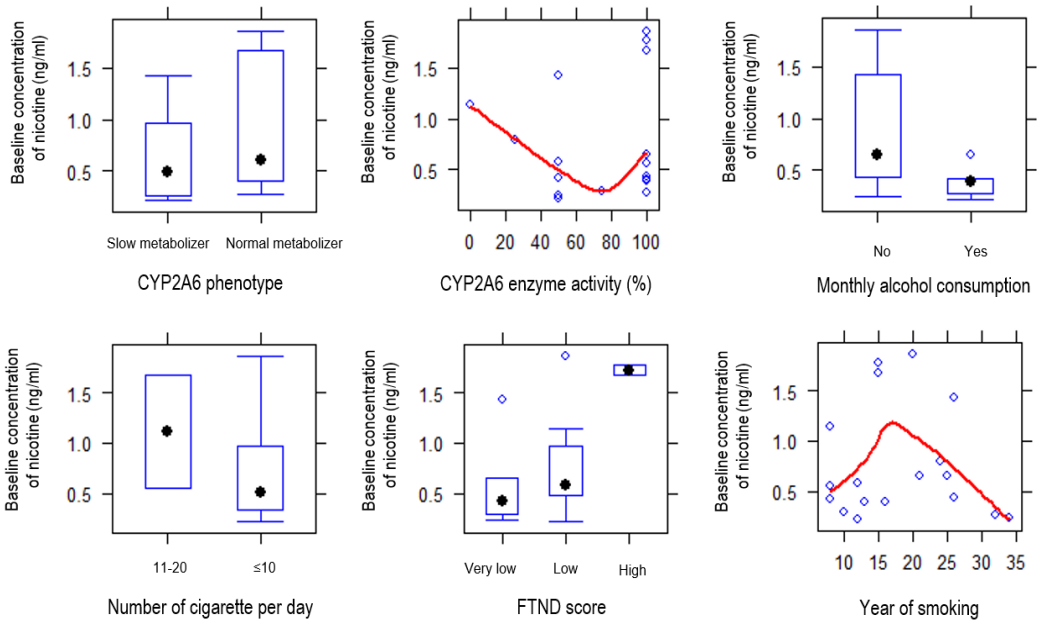

Figure 13 Plots of the relationship between covariate values and baseline concentration of nicotine

Table 10 Changes in OFV during forward addition step 1

Parameter	Added Covariate	Model	OFV	ΔOFV
		Base	-143.931	
CL/F	CYP2A6 phenotype (add shift, df=1)	CL/F=θ1+θ5*PHENO	-151.775	-7.844*
	CYP2A6 activity(L)	CL/F= θ1+θ5*(CYP2A6-100)	-161.165	-17.234*
	CYP2A6 activity(P)	CL/F= θ1+(CYP2A6/100)**θ5	-155.919	-11.988*
	CYP2A6 activity(E)	CL/F= θ1+EXP((CYP2A6/100)*θ5)	-158.270	-14.339*
	CPD (add shift, df=1)	CL/F= θ1+θ5*NSMOKE	-147.701	-3.77
	FTND score (add shift, df=2)	CL/F=θ1+θ5*LOW+ θ6*HIGH	-148.710	-4.799
	Year of smoking(L)	CL//F= θ1+θ5*(YSMOKE-15.5)	-144.165	-0.234
	Year of smoking (P)	CL/F= θ1+(YSMOKE/15.5)**θ5	-144.301	-0.37
	Year of smoking (E)	CL/F= θ1+EXP((YSMOKE/15.5)*θ5)	-144.149	-0.218
	Monthly alcohol consumption (add shift, df=1)	CL= θ1+θ5*ALCOHOL	-144.661	-0.73
V/F	BW(L)	V/F= θ2+θ5*(BW-70.5)	-145.208	-1.277
	BW(P)	V/F= θ2+(BW/70.5)**θ5	-145.130	-1.199
	BW(E)	V/F= θ2+EXP((BW/70.5)*θ5)	NA[a]	
	ABW(L)	V/F= θ2+θ5*(ABW-68.5)	-145.026	-1.095
	ABW(P)	V/F= θ2+(ABW/68.5)**θ5	-145.026	-1.095
	ABW(E)	V/F= θ2+EXP((ABW/68.5)*θ5)	-145.092	-1.161
	IBW(L)	V/F= θ2+θ5*(IBW-65.5)	-144.121	-0.19
	IBW(P)	V/F= θ2+(IBW/65.5)**θ5	-144.123	-0.192
	IBW(E)	V/F= θ2+EXP((IBW/65.5)*θ5)	NA[a]	
	BMI(L)	V/F= θ2+θ5*(BMI-24.6)	-145.162	-1.231
	BMI(P)	V/F= θ2+(BMI/24.6)**θ5	-145.068	-1.137
	BMI(E)	V/F= θ2+EXP((BMI/24.6)*θ5)	NA[a]	
	Obesity	V/F= θ2+θ5*OBESE	-144.017	-0.086
MTT	CYP2A6 phenotype	MTT=θ3+θ5*PHENO	-144.708	-0.777
	CYP2A6 activity(L)	MTT = θ3+θ5*(CYP2A6-100)	NA[a]	
	CYP2A6 activity(P)	MTT = θ3+(CYP2A6/100)**θ5	NA[b]	
	CYP2A6 activity(E)	MTT=θ3+EXP((CYP2A6/100)*θ5)	-146.282	-2.352

Table 10 Changes in OFV during forward addition step 1

Parameter	Added Covariate	Model	OFV	ΔOFV
		Base	-143.931	
C_0	CYP2A6 phenotype (add shift, df=1)	C_0 = θ1+θ5*PHENO	-145.143	-1.212
	CYP2A6 activity(L)	C_0 = θ1+θ5*(CYP2A6-100)	NA[b]	
	CYP2A6 activity(P)	C_0 = θ1+(CYP2A6/100)**θ5	-95.352	+48.579
	CYP2A6 activity(E)	C_0 = θ1+EXP((CYP2A6/100)*θ5)	-144.043	-0.112
	Number of cigarettes (add shift, df=1)	C_0 = θ1+θ5*NSMOKE	-145.132	-1.201
	FTND score (add shift, df=2)	C_0 =θ1+θ5*LOW+ θ6*HIGH	NA[a]	
	Year of smoking(L)	C_0 = θ1+θ5*(YSMOKE-15.5)	NA[a]	
	Year of smoking (P)	C_0 = θ1+(YSMOKE/15.5)**θ5	-143.992	-0.061
	Year of smoking (E)	C_0 = θ1+EXP((YSMOKE/15.5)*θ5)	-144.283	-0.352
	Monthly alcohol consumption (add shift, df=1)	C_0 = θ1+θ5*ALCOHOL	-149.107	-5.176*

*A decrease in OFV ≥ 3.84 indicates that the covariate has a significant effect on the PK parameter (p-Value<0.05). NA[b], Covariance step was not successful. L, linear model; P, power model; E, exponential model. Add shift, additive shift model; df= degree of freedom

Table 11 Changes in OFV during forward addition step 2

Parameter	Added CoVariate	Model	OFV	ΔOFV
Base model added with enzymatic activity of CYP2A6 genotypes		CL= θ1+θ5*(CYP2A6-100)	-161.165	
C0	Monthly alcohol consumption	CL= θ1+θ5*(CYP2A6-100) C0= θ4+θ6*ALCOHOL	-166.764	-5.599*

*A decrease in OFV ≥ 3.84 indicates that the covariate has a significant effect on the PK parameter (p-Value<0.05).

Table 12 Changes in OFV during backward deletion of full model

Parameter	Removed Covariate	Model	OFV	ΔOFV
Full model		CL= θ1+θ5*(CYP2A6-100) C0= θ4+θ6*ALCOHOL	-166.764	
CL/F	Enzymatic activity of CYP2A6 genotypes	C0= θ4+θ6*ALCOHOL	-149.107	17.639*
C0	Monthly alcohol consumption	CL= θ1+θ5*(CYP2A6-100)	-161.165	5.599

*An increase in OFV ≥ 10.83 indicates that the covariate has a significant effect on the PK parameter (p-Value<0.001).

Table 13 Population PK parameter estimates of base model, final model and bootstrap

Parameter	Base Model Estimate [%RSE]	Final Model Estimate [%RSE]	Bootstrap (n=991)		Shrinkage of final model (%)
			Bootstrap medium	95% CI	
Fixed effect					
CL/F = TVCL + θ_{CYP2A6} * (CYP2A6-100)					
TVCL (L/h)	190 [14.9]	266 [10.7]	271	219 - 348	-
θ_{CYP2A6}	-	2.34 [12.6]	2.37	1.08 - 3.56	-
V/F (L)	856 [10.3]	851 [10.3]	863	703 - 1050	-
KA (h^{-1})	2.9 *fix*	2.9 *fix*	-	-	-
MTT(h)	0.12 [15.8]	0.12 [15.6]	0.12	0.08 - 0.17	-
C0 (ng/ml)	0.58 [19.9]	0.57 [18.8]	0.59	0.39 - 0.85	-
Random effect (CV%)					
IIV of CL/F	64.9 [43.5]	38.5 [43.3]	37.1	14.8 - 53.9	3.9
IIV of V/F	40.4 [32.8]	38.1 [29.7]	37.2	23.7 - 49.4	4.8
IIV of MTT	51.6 [29.2]	54.1 [34.0]	53.5	0.2 - 87.0	15.2
IIV of C0	72.5 [23.0]	73.1 [22.6]	73.2	50.7 - 96.1	4.2
RUV	14.7 [24.7]	14.7 [26.3]	14.8	10.5 -18.4	18.2
Secondary parameters		**Median (minimum-maximum)**			
C_{max} (ng/ml)		1.8 (1.1-4.5)			
t_{max} (h)		1.5 (1.0-2.0)			
AUC_{0-6} (h*ng/ml)		6.6 (3.1-21.4)			
AUC_{0-inf} (h*ng/ml)		8.7 (3.3-57.2)			
$t_{½}$ (h)		2.9 (1.3-7.9)			

RSE, relative standard error; CI, confidence interval; TVCL, typical value of apparent elimination clearance; CL/F, apparent elimination clearance; V/F, apparent volume of distribution; Ka, first-order absorption rate constant; MTT, mean transit time; C0, baseline concentration; CV, coefficient of variation; IIV, inter-individual variability; RUV, residual unexplained variability; $C_{max,}$ maximum plasma concentration, $t_{max,}$ time to reach C_{max} ; AUC_{0-6}, area under plasma concentration-time curve from start of product use to last sampling time; AUC_{0-inf} ,area under plasma concentration-time curve from start of product use extrapolated to infinity; $t_{½}$ elimination half-life.

4.4 Model evaluation

Parameter estimates are presented in table 13. Fixed effect parameters were estimated with high precision with relative standard errors (%RSEs) between 10% and 20%. The goodness-of-fit plots did not show any obvious model misspecification (figure 14). However, a small deviation was found in higher concentrations, which was contributed by substantially higher plasma concentrations of subject who has the complete lack of CYP2A6 enzyme activity. Final parameter estimates of the model was within 95% confidence interval of the range of estimated obtained from 991 successful bootstrap runs (out of 1,000), which indicated a stable and appropriate model (table 13). Value of eta and epsilon shrinkage were within acceptable limits (3.9-18.2%). Prediction- corrected visual predictive checks are presented in figure 15 showing a good predictive performance of the model.

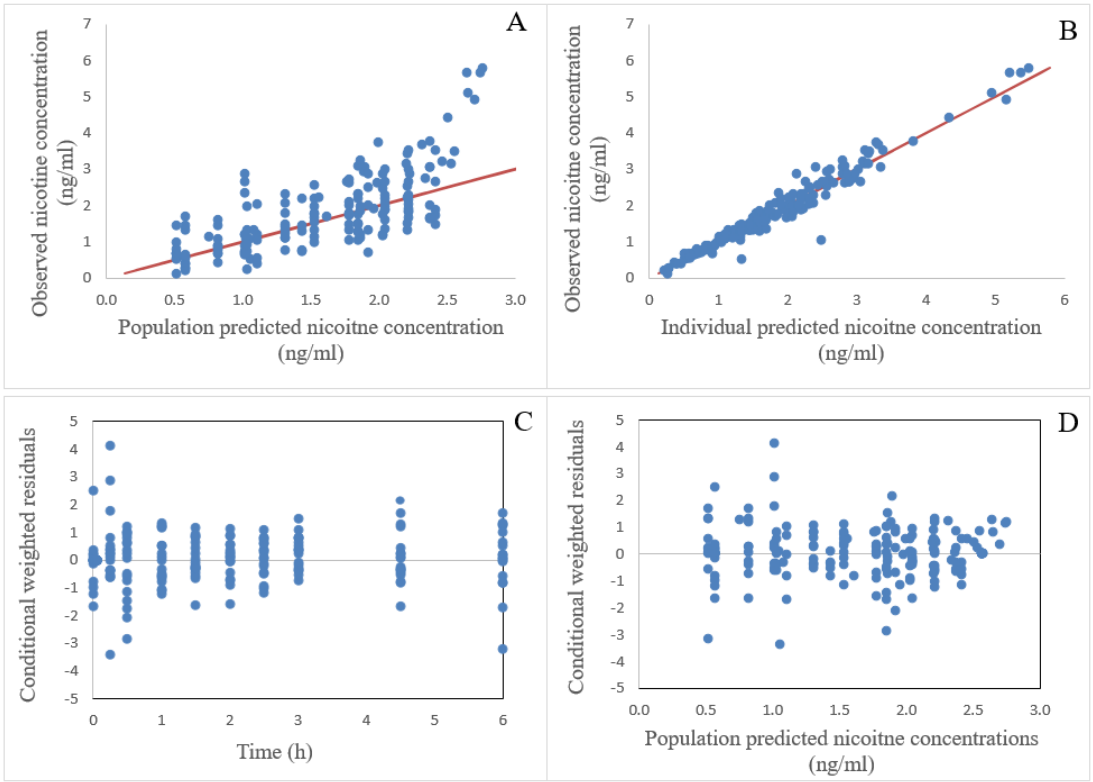

Figure 14 Basic goodness-of-fit plots of final model

A. observed vs. population predicted nicotine concentrations, B. observed vs. individual predicted nicotine concentrations, C. conditional weighted residuals vs. time, D. conditional weighted residuals vs. population predicted nicotine concentrations

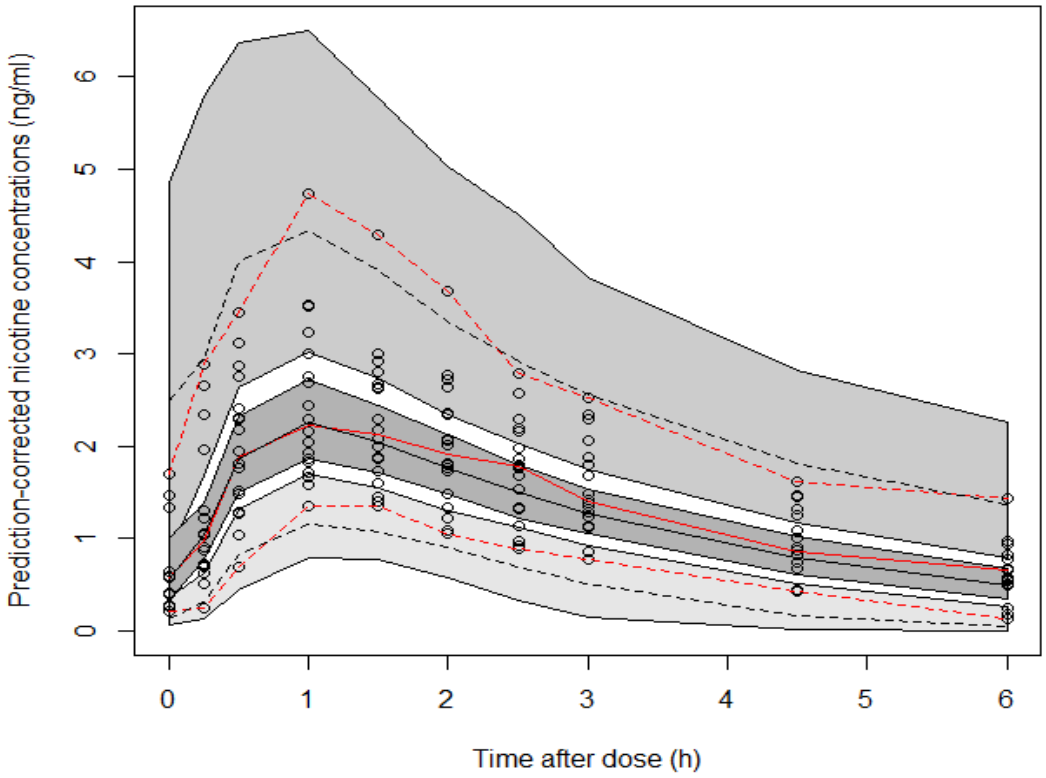

Figure 15 Prediction-corrected visual predictive check of final model.

Open circles represent the prediction-corrected observed concentrations of nicotine. Black dashed line at the top, black solid line, and black dashed line at the bottom represent 97.5th, 50th and 2.5th predicted percentiles respectively. Observed 97.5th, 2.5th and 50th percentiles are presented as red dashed lines and red solid line. Shaded areas represent 95% prediction interval.

CHAPTER V DISCUSSION & CONCLUSION

This study is the first to report the population pharmacokinetics of nicotine following nicotine gum administration in a Thai population showing that the enzymatic activity of different CYP2A6 genotypes significantly affect the clearance of nicotine. Pharmacokinetic parameters resulted from this study might be useful for the development of individualized smoking cessation therapy.

Direct comparison with previous population pharmacokinetic study of nicotine might be difficult because of different study design. Marchand developed population pharmacokinetics of nicotine following different kinds of NRTs (2-mg nicotine gum and 1-mg nicotine nasal spray) and tobacco products (tobacco heating system and cigarette) administration[25]. A total of 16 blood samples over 24-hour (9 points within 1st hour) was collected per subject in Marchand study, whereas a total of 10 blood samples over 6-hour (4 points within 1st hour) was collected in the present study.

Two-compartment model best described the observed data in Marchand study, whereas one-compartment did well in the present study. Longer blood collection period (24 hours) in Marchand study might contribute the discrepancy in the number of distribution compartments between two studies. In the present model, a fist-order absorption with 6 transit compartments model was found to provide the best fit of the observed data, whereas a zero-order absorption model did well in Marchand study. Marchand tested only zero- and first- order absorption models and did not investigate a transit compartment model. This might partly explain the discrepancy in absorption model between studies.

Population PK parameter estimates of nicotine for a typical male subject with 100% CYP2A6 enzymatic activities from two population pharmacokinetic studies were compared in table 14. Oral elimination clearance (CL/F) of nicotine in the present study was 266 L/h, which is approximately 7 times higher than that in Marchand study. Apparent volume of distribution of nicotine (V/F) in the present study was 851L, which was also 3 times higher than that in Marchand study. Moreover, CL/F

and V/F in this study were higher compared to most values found in non-compartmental analyses of nicotine gum[13-18] (CL/F=54.7-178.6 L/h and V/F=322.1-833.0 L), but it should be noted that none of NCAs included data on CYP2A6 polymorphism. Elimination half-life of nicotine in the present study (2.20 h) is consistent with the value reported in literature. These high CL/F and V/F could suggest low bioavailability in Thai population. It is worth noted that different brands of 2-mg nicotine gum were used in this study and previous studies (Nicorette)[13-18, 25].

Regarding exposure of nicotine, it is interesting that nicotine exposure after chewing nicotine gum is substantially low in Thai population compared to non-Thai population. Comparison of pharmacokinetic parameters of plasma nicotine from both compartmental and non-compartmental analysis of single-dose 2mg nicotine gum in non-Thai population versus Thai population was shown in table 15. Model predicted $AUC_{0\text{-}inf}$ of nicotine after single dose 2mg nicotine gum administration in the current study is 8.7 h*ng/ml, which is less than the lower range of $AUC_{0\text{-}inf}$ values found in non-Thai population ($AUC_{0\text{-}inf}$ = 11.3-31.1 h*ng/ml). This observation might challenge the therapeutic efficacy of current dosage regimen of nicotine gum for Thai population. Therefore, the efficacy of current dosage regimen should be confirmed by further studies. Moreover, studies with larger sample size and more frequent sampling design is recommended to better characterize the pharmacokinetics of nicotine gum.

Base model consisted of a component describing the disposition of pre-dose concentrations. Despite 12-hour washout period, plasma concentrations of nicotine were measurable before dosing. The presence of pre-dose concentrations could interfere with the estimation of the pharmacokinetic parameters of nicotine. Among different approaches to handle the baseline data, estimating the typical value and IIV of baseline concentrations provided the best performance, with less bias and less imprecision compared to other methods[65]. Therefore, typical value and IIV of pre-dose concentrations were estimated in this study. Then, pre-dose concentrations were modelled as mono-exponential decay as described in literature[25]. Addition of pre-dose model into the base model provided the better model fit in every aspects of absorption models and RUV models. Estimated baseline concentration in this

study (0.58 ng/ml) is somewhat higher than that in Marchand study. Shorter washout period in this study (12h) compared to Marchand study (24h) might explain the difference in estimated baseline value between studies.

CYP2A6 enzyme activity was significant covariate on CL/F of nicotine. CYP2A6 enzyme is major metabolizing enzyme of nicotine and CYP2A6 polymorphism has a significant impact on metabolism of nicotine[8]. Different CYP2A6 genetic variants results in variation in CYP2A6 enzyme activity, which in turn affects the rate of nicotine metabolism[19]. According to final model described in equation 2, if the CYP2A6 activity decreased 25%, the CL/F decreased by 58.5 L/h (or 22%). Positive relationship between CL/F of nicotine and CYP2A6 activity was consistent with previously reported data[8, 25]. However, interpretation of CYP2A6 activity between studies should be cautious because different methods of CYP2A6 activity measurement might affect the results. Nicotine metabolite ratio (NMR) has been reported a valid indicator of CYP2A6 activity. Unfortunately, NMR data were not available and we used activity score system to predict CYP2A6 activity based on known enzymatic activity of CYP2A6 variants. However, it is worth noted that activity score system is also a valid, easy-to-use tool to predict phenotype and is utilized to provide genotype-based dosing recommendation in clinical settings[56, 58].

Two studies reported that there was an association between alcohol consumption and rate of nicotine metabolism[44, 45]. Therefore, the effect of monthly alcohol consumption on clearance of nicotine was investigated, but significant pharmacokinetic relation was not found. Chronic heavy drinking was found in subjects recruited in previous two studies and they were diagnosed with alcohol dependent disorder[44, 45]. Meanwhile, 5 out of 18 individuals in the current study drunk between 5 and 50 glasses of alcohol containing beverages on a monthly basis and alcohol dependent disorders were not found in these subjects. The lack of patients with heavy drinking and alcohol dependent disorder in the present study might explain why alcohol consumption was not found to be a significant covariate in the present study.

There are some limitations in this study. First, this was a retrospective analysis performed on secondary data with a small sample size. Only Thai smokers were

included in the study. Therefore, the results from this study might not represent other populations. Second, all subjects were male. So, the differences in pharmacokinetic parameters of nicotine between male and female could not be investigated in this study. However, it is worth noted that the prevalence of smoking is about 15-20 times higher among men than women in Thailand[68]. Finally, 6-hour sampling time are relatively short and might affect the characterization of the elimination phase.

In conclusion, this first report on the population pharmacokinetics of nicotine gum in Thai population provided the pharmacokinetic model of nicotine gum, which was best described by a linear one-compartment disposition model with first-order absorption and 6 transit compartments. It was found that enzymatic activity of different CYP2A6 genotypes influences the clearance of nicotine. Providing personalized smoking cessation therapy based on CYP2A6 genetic variation is important to optimize therapeutic efficacy of nicotine medications. Findings of this study could help provide individualized dosing regimen in order to increase the rate of successful quit.

Table 14 Population PK parameter estimates of nicotine gum

Parameter (unit)	Marchand (2017)	Present study
	Two-compartment with 1st order elimination	One-compartment with 1st order elimination
$t_{1/2}$ (h)	0.8 distribution $t_{1/2}$, 11.97 elimination $t_{1/2}$	2.90 elimination $t_{1/2}$
CL/F (L/h)	36.3	266.0
V1/F (L)	76.7	851.0
V2/F (L)	171.0	-
Q/F (L/h)	10.3	-
	Zero-order absorption	1st–order absorption with 6 transit compartments
T_{dur} (h)	0.75	-
MTT(h), K_a(h^{-1})	-	0.12, 2.90
C0 (ng/ml)	0.36	0.58

$t_{1/2}$, half-life T_{dur} duration of zero-order absorption; MTT mean transit time; C0 pre-dose concentration

Table 15 Comparison of pharmacokinetic parameters of plasma nicotine after administration of single dose 2mg nicotine gum in non-Thai population versus Thai population.

	NCA									POP PK	
Author (year)	Choi (2003) [a]	Dautzenberg (2007)	Muneesh (2016)	Hansson (2017) [a]	Brossard (2017) [b]		Du (2018)	Original clinical trial		Marchand (2017) [b]	This study
Population	American	French	Indian	Swedish	Japanese		European	Thai		Japanese	Thai
N	23	9	43	44	18 (Tokyo)	18 (Saitama)	62	10 (normal metabolizer)	8 (Slow metabolizer)	36	18
Blood sampling	14 samples over 12 h	11 samples over 8 h	19 samples over 24 h	19 samples over 12 h	16 samples over 24 h		13 samples over 12 h	10 samples over 6 h		16 samples over 24 h	10 samples over 6 h
C_{max} (ng/ml)	4.0 ± 1.5	2.9 ± 1.2	7.3 ± 2.1	5.9 ± 1.9	4.8	7.52	3.7 ± 1.3	2.53 ± 1.80	3.15 ± 0.47	5.7	1.8 (1.1-4.5)
t_{max} (h)	0.8 ± 0.2	0.8 ± 0.1	0.7 (0.3,3.0)	0.5 [c]	0.6 [d]	0.8 [d]	0.8 (0.5, 1.5)	0.80 ± 0.13	1.50 ± 0.13	0.8	1.5 (1.0-2.0)
$AUC_{0\text{-last}}$ (h*ng/ml)	10.7 ± 6.6	10.6 ± 4.4	32.3 ± 11.5	15.1 ± 5.3	14.9	27.9	10.2 ± 3.78	8.30 ± 0.77	13.32 ± 2.45	21.3	6.6 (3.1-21.4)
$AUC_{0\text{-inf}}$ (h*ng/ml)	11.3 ± 7.6	13.8 ± 5.6	36.6 ± 13.4	17.1 ± 6.0	16.6	31.1	11.2 ± 4.0	11.23 ± 1.41	24.63 ± 7.03	27.0	8.7 (3.3-57.2)
$t_{1/2}$ (h)	2.5 ± 1.2	2.5 ± 1.0	7.4 ± 4.7	2.9 [c]	4.8	3.5	2.0 (1.2, 4.2)	2.59 ± 0.30	4.22 ± 0.63	0.8*, 11.97	2.9 (1.3-7.9)

Values were expressed as Mean ± SD or Median (minimum, maximum). NCA non-compartmental pharmacokinetic analysis; POP PK population pharmacokinetic analysis; C_{max} maximum plasma concentration of nicotine; t_{max} time to C_{max}; $AUC_{0\text{-last}}$ area under plasma concentration-time curve from start of product use to last sampling time; $AUC_{0\text{-inf}}$ area under plasma concentration-time curve from start of product use extrapolated to infinity;

$t_{1/2}$ plasma elimination half-life; *distribution half-life.

[a] Plasma concentrations of nicotine were reported as baseline-adjusted values because of measurable pre-dose concentrations.

[b] Values were expressed as geometric mean.

[c] Value was expressed as mean. Standard deviation was not reported.

[d] Value was expressed as median. Range was not reported.

REFERENCES

1. National Center for Chronic Disease Prevention and Health Promotion Office on Smoking and Health. Reports of the Surgeon General. The Health Consequences of Smoking-50 Years of Progress: A Report of the Surgeon General. Atlanta (GA): Centers for Disease Control and Prevention (US); 2014.
2. World Helath Organization. WHO report on the global tobacco epidemic Geneva [cited 2019 Mar 11]. Available from: https://www.who.int/tobacco/global_report/2017/executive-summary/en/.
3. World Health Organization. Heart disease and stroke are one of the commonest ways by which tobacco kills people. New Delhi: WHO in South-East Asia; 2018 [cited 2019 July 17]. Available from: http://www.who.int/iris/bitstream/10665/272690/1/wntd_2018_thailand_fs.pdf.
4. Zhao J, Pachanee CA, Yiengprugsawan V, Seubsman SA, Sleigh A. Smoking, smoking cessation, and 7-year mortality in a cohort of Thai adults. Popul Health Metr. 2015;13:30.
5. Hartmann-Boyce J, Chepkin SC, Ye W, Bullen C, Lancaster T. Nicotine replacement therapy versus control for smoking cessation. Cochrane Database Syst Rev. 2018;5:Cd000146.
6. Chinwong S, Chinwong D. A national survey of community pharmacists on smoking cessation services in Thailand. Pharmacy (Basel). 2018;6(3):101.
7. Benowitz NL, Hukkanen J, Jacob P, 3rd. Nicotine chemistry, metabolism, kinetics and biomarkers. Handb Exp Pharmacol. 2009(192):29-60.
8. Hukkanen J, Jacob P, 3rd, Benowitz NL. Metabolism and disposition kinetics of nicotine. Pharmacol Rev. 2005;57(1):79-115.
9. McKinney DL, Vansickel AR. Nicotine chemistry, pharmacology, and pharmacokinetics. Neuropathology of Drug Addictions and Substance Misuse. 1: Elsevier; 2016. p. 93-103.
10. Russell MA, Feyerabend C, Cole PV. Plasma nicotine levels after cigarette smoking and chewing nicotine gum. Br Med J. 1976;1(6017):1043-6.

11. McNabb ME, Ebert RV, McCusker K. Plasma nicotine levels produced by chewing nicotine gum. Jama. 1982;248(7):865-8.
12. Benowitz NL, Jacob P, 3rd, Savanapridi C. Determinants of nicotine intake while chewing nicotine polacrilex gum. Clin Pharmacol Ther. 1987;41(4):467-73.
13. Choi JH, Dresler CM, Norton MR, Strahs KR. Pharmacokinetics of a nicotine polacrilex lozenge. Nicotine Tob Res. 2003;5(5):635-44.
14. Dautzenberg B, Nides M, Kienzler JL, Callens A. Pharmacokinetics, safety and efficacy from randomized controlled trials of 1 and 2 mg nicotine bitartrate lozenges (Nicotinell). BMC Clin Pharmacol. 2007;7:11.
15. Garg M, Naidu R, Iyer K, Jadhav R. Bioequivalence of two different nicotine chewing gum formulations of two different strengths (2 mg and 4 mg) in Indian healthy adult human male smoker subjects. J Bioequiv Availab. 2016;8:074-9.
16. Brossard P, Weitkunat R, Poux V, Lama N, Haziza C, Picavet P, et al. Nicotine pharmacokinetic profiles of the Tobacco Heating System 2.2, cigarettes and nicotine gum in Japanese smokers. Regul Toxicol Pharmacol. 2017;89:193-9.
17. Hansson A, Rasmussen T, Kraiczi H. Single-dose and multiple-dose pharmacokinetics of nicotine 6 mg gum. Nicotine Tob Res. 2017;19(4):477-83.
18. Du D. A single-dose, crossover-design bioequivalence study comparing two nicotine gum formulations in healthy subjects. Adv Ther. 2018;35(8):1169-80.
19. Tanner JA, Tyndale RF. Variation in CYP2A6 activity and personalized medicine. J Pers Med. 2017;7(4):18.
20. Lerman C, Jepson C, Wileyto EP, Patterson F, Schnoll R, Mroziewicz M, et al. Genetic variation in nicotine metabolism predicts the efficacy of extended-duration transdermal nicotine therapy. Clini Pharmaco Ther. 2010;87(5):553-7.
21. Chen LS, Bloom AJ, Baker TB, Smith SS, Piper ME, Martinez M, et al. Pharmacotherapy effects on smoking cessation vary with nicotine metabolism gene (CYP2A6). Addiction. 2014;109(1):128-37.
22. Schnoll RA, Patterson F, Wileyto EP, Tyndale RF, Benowitz N, Lerman C. Nicotine metabolic rate predicts successful smoking cessation with transdermal nicotine: A validation study. Pharmacol Biochem Behav. 2009;92(1):6-11.
23. Lerman C, Tyndale R, Patterson F, Wileyto EP, Shields PG, Pinto A, et al. Nicotine

metabolite ratio predicts efficacy of transdermal nicotine for smoking cessation. Clini Pharmaco Ther. 2006;79(6):600-8.

24. Ho M, Mwenifumbo J, Al Koudsi N, Okuyemi K, Ahluwalia J, Benowitz N, et al. Association of nicotine metabolite ratio and CYP2A6 genotype with smoking cessation treatment in African-American light smokers. Clini Pharmaco Ther. 2009;85(6):635-43.
25. Marchand M, Brossard P, Merdjan H, Lama N, Weitkunat R, Ludicke F. Nicotine population pharmacokinetics in healthy adult smokers: A retrospective analysis. Eur J Drug Metab Pharmacokinet. 2017;42(6):943-54.
26. Bundhamcharoen K, Aungkulanon S, Makka N, Shibuya K. Economic burden from smoking-related diseases in Thailand. Tob Control. 2016;25(5):532-7.
27. Benowitz NL. Clinical pharmacology of nicotine: Implications for understanding, preventing, and treating tobacco addiction. Clin Pharmacol Ther. 2008;83(4):531-41.
28. MIMS. Nicotine 2019 [cited 2019 March 27]. Available from: http://www.mims.com/thailand/drug/search?q=nicotine&page=1.
29. Wadgave U, Nagesh L. Nicotine Replacement Therapy: An Overview. Int J Health Sci (Qassim). 2016;10(3):425-35.
30. Molyneux A. Nicotine replacement therapy. BMJ (Clinical research ed). 2004;328(7437):454-6.
31. Henningfield JE, Fant RV, Buchhalter AR, Stitzer ML. Pharmacotherapy for nicotine dependence. CA Cancer J Clin. 2005;55(5):281-99; quiz 322-3, 5.
32. Henningfield JE, Keenan RM. Nicotine delivery kinetics and abuse liability. J Consult Clin Psychol. 1993;61(5):743-50.
33. Mendelsohn C. Optimising nicotine replacement therapy in clinical practice. Aust Fam Physician. 2013;42:305-9.
34. Ells AW, Sherman J. Community and Clinical Pharmacy Services: A step by step approach: McGraw-Hill Education; 2013.
35. Lindson N, Chepkin SC, Ye W, Fanshawe TR, Bullen C, Hartmann-Boyce J. Different doses, durations and modes of delivery of nicotine replacement

therapy for smoking cessation. Cochrane Database Syst Rev. 2019(4).

36. Shiffman S, Sembower MA, Rohay JM, Gitchell JG, Garvey AJ. Assigning dose of nicotine gum by time to first cigarette. Nicotine Tob Res. 2013;15(2):407-12.

37. Micromedex® I. Nicorette gum [Available from: https://www.micromedexsolutions.com/micromedex2/librarian/CS/C89688/ND_PR/evidencexpert/ND_P/evidencexpert/DUPLICATIONSHIELDSYNC/308FAF/ND_PG/evidencexpert/ND_B/evidencexpert/ND_AppProduct/evidencexpert/ND_T/evidencexpert/PFActionId/evidencexpert.IntermediateToDocumentLink?docId=00001763&contentSetId=128&title=Nicorette+Gum&servicesTitle=Nicorette+Gum.

38. Hall SM, Humfleet GL, Munoz RF, Reus VI, Robbins JA, Prochaska JJ. Extended treatment of older cigarette smokers. Addiction. 2009;104(6):1043-52.

39. Svensson CK. Clinical pharmacokinetics of nicotine. Clin Pharmacokinet. 1987;12(1):30-40.

40. Henningfield JE. Nicotine medications for smoking cessation. N Engl J Med. 1995;333(18):1196-203.

41. Molyneux A. Nicotine replacement therapy. Bmj. 2004;328(7437):454-6.

42. Taghavi T, St Helen G, Benowitz NL, Tyndale RF. Effect of UGT2B10, UGT2B17, FMO3, and OCT2 genetic variation on nicotine and cotinine pharmacokinetics and smoking in African Americans. Pharmacogenet Genomics. 2017;27(4):143-54.

43. Linakis MW, Rower JE, Roberts JK, Miller EI, Wilkins DG, Sherwin CMT. Population pharmacokinetic model of transdermal nicotine delivered from a matrix-type patch. Br J Clin Pharmacol. 2017;83(12):2709-17.

44. Gubner NR, Kozar-Konieczna A, Szoltysek-Boldys I, Slodczyk-Mankowska E, Goniewicz J, Sobczak A, et al. Cessation of alcohol consumption decreases rate of nicotine metabolism in male alcohol-dependent smokers. Drug Alcohol Depend. 2016;163:157-64.

45. Dermody SS, Hendershot CS, Andrade AK, Novalen M, Tyndale RF. Changes in nicotine metabolite ratio among daily smokers receiving treatment for alcohol use disorder. Nicotine Tob Res [Internet]. 2018 Dec 17.

46. Benowitz NL, Jacob P, 3rd. Nicotine and cotinine elimination pharmacokinetics in smokers and nonsmokers. Clin Pharmacol Ther. 1993;53(3):316-23.

47. Lee BL, Benowitz NL, Jacob P, 3rd. Influence of tobacco abstinence on the disposition kinetics and effects of nicotine. Clin Pharmacol Ther. 1987;41(4):474-9.

48. Mahavorasirikul W, Tassaneeyakul W, Satarug S, Reungweerayut R, Na-Bangchang C, Na-Bangchang K. CYP2A6 genotypes and coumarin-oxidation phenotypes in a Thai population and their relationship to tobacco smoking. Eur J Clin Pharmacol. 2009;65(4):377-84.

49. Kumondai M, Hosono H, Orikasa K, Arai Y, Arai T, Sugimura H, et al. Genetic polymorphisms of CYP2A6 in a case-control study on bladder cancer in Japanese smokers. Biol Pharm Bull. 2016;39(1):84-9.

50. Ariyoshi N, Miyamoto M, Umetsu Y, Kunitoh H, Dosaka-Akita H, Sawamura Y, et al. Genetic polymorphism of CYP2A6 gene and tobacco-induced lung cancer risk in male smokers. Cancer Epidemiol Biomarkers Prev. 2002;11(9):890-4.

51. Wang C, Wang P, Yang LP, Pan J, Yang X, Ma HY. Association of CYP2C9, CYP2A6, ACSM2A, and CPT1A gene polymorphisms with adverse effects of valproic acid in Chinese patients with epilepsy. Epilepsy Res. 2017;132:64-9.

52. Zhao M, Zhang T, Li G, Qiu F, Sun Y, Zhao L. Associations of CYP2C9 and CYP2A6 Polymorphisms with the Concentrations of Valproate and its Hepatotoxin Metabolites and Valproate-Induced Hepatotoxicity. Basic Clin Pharmacol Toxicol. 2017;121(2):138-43.

53. Ruwali M, Pant MC, Shah PP, Mishra BN, Parmar D. Polymorphism in cytochrome P450 2A6 and glutathione S-transferase P1 modifies head and neck cancer risk and treatment outcome. Mutat Res. 2009;669(1-2):36-41.

54. Dempsey D, Tutka P, Jacob P, 3rd, Allen F, Schoedel K, Tyndale RF, et al. Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. Clin Pharmacol Ther. 2004;76(1):64-72.

55. Gaedigk A. Chapter 2. Genetic concepts of pharmacogenomics: Basic review of DNA, genes, polymorphisms, haplotypes and nomenclature. In: Bertino JS, DeVane CL, Fuhr U, Kashuba AD, Ma JD, editors. Pharmacogenomics: An introduction and clinical perspective. New York, NY: The McGraw-Hill Companies; 2013.

56. Gaedigk A, Simon SD, Pearce RE, Bradford LD, Kennedy MJ, Leeder JS. The CYP2D6 activity score: translating genotype information into a qualitative measure of phenotype. Clin Pharmacol Ther. 2008;83(2):234-42.

57. Arab-Alameddine M, Di Iulio J, Buclin T, Rotger M, Lubomirov R, Cavassini M, et al. Pharmacogenetics-based population pharmacokinetic analysis of efavirenz in HIV-1-infected individuals. Clini Pharmaco Ther. 2009;85(5):485-94.

58. Hicks JK, Sangkuhl K, Swen JJ, Ellingrod VL, Muller DJ, Shimoda K, et al. Clinical pharmacogenetics implementation consortium guideline (CPIC) for CYP2D6 and CYP2C19 genotypes and dosing of tricyclic antidepressants: 2016 update. Clin Pharmacol Ther. 2017;102(1):37-44.

59. Ghosheh OA, Browne D, Rogers T, de Leon J, Dwoskin LP, Crooks PA. A simple high performance liquid chromatographic method for the quantification of total cotinine, total 3'-hydroxycotinine and caffeine in the plasma of smokers. J Pharm Biomed Anal. 2000;23(2-3):543-9.

60. Beal S, Sheiner L, Boeckmann A, Bauer R. NONMEM Users Guides. 1989-2011. Icon Development Solutions, Ellicott City, Maryland, USA. 2011;1(1):1.

61. Jonsson EN, Karlsson MO. Xpose--an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. Comput Methods Programs Biomed. 1999;58(1):51-64.

62. Pétricoul O, Cosson V, Fuseau E, Marchand M. Population models for drug absorption and enterohepatic recycling. CPT Pharmacometrics Syst Pharmacol. 2007:345-82.

63. Savic RM, Jonker DM, Kerbusch T, Karlsson MO. Implementation of a transit compartment model for describing drug absorption in pharmacokinetic studies. J Pharmacokinet Pharmacodyn. 2007;34(5):711-26.

64. Mavroudis PD, Kosmidis K, Macheras P. On the unphysical hypotheses in pharmacokinetics and oral drug absorption: Time to utilize instantaneous rate coefficients instead of rate constants. Eur J Pharm Sci. 2019;130:137-46.

65. Dansirikul C, Silber HE, Karlsson MO. Approaches to handling pharmacodynamic baseline responses. J Pharmacokinet Pharmacodyn. 2008;35(3):269-83.

66. Bergstrand M, Hooker AC, Wallin JE, Karlsson MO. Prediction-corrected visual

predictive checks for diagnosing nonlinear mixed-effects models. Aaps J. 2011;13(2):143-51.

67. Nguyen TH, Mouksassi MS, Holford N, Al-Huniti N, Freedman I, Hooker AC, et al. Model Evaluation of Continuous Data Pharmacometric Models: Metrics and Graphics. CPT Pharmacometrics Syst Pharmacol. 2017;6(2):87-109.

68. Chinwong D, Mookmanee N, Chongpornchai J, Chinwong S. A comparison of gender differences in smoking behaviors, intention to quit, and nicotine dependence among Thai university students. J Addict. 2018; 2018:8. doi: 10.1155/2018/8081670.

APPENDIX

APPENDIX A: NONMEM code for final model

```
$PROBLEM Final model of nicotine
$INPUT C ID TIME AMT DV LGDV EVID MDV CMT AGE WT HEIGHT IBW ABW BMI BSA
CYPACTIVITY OBESE TWOPHENO SEVENGENO THREEGENO ALCOHOL YSMOKE
YSMOKEC NSMOKE FTND
$DATA NICOTINE.CSV IGNORE=C
$SUBROUTINES ADVAN6 TOL=3
$MODEL
  COMP= (1); DOSE COMPARTMENT
  COMP= (2); CENTRAL COMPARTMENT
  COMP= (3); TRANSIT COMPARTMENT 1
  COMP= (4); TRANSIT COMPARTMENT 2
  COMP= (5); TRANSIT COMPARTMENT 3
  COMP= (6); TRANSIT COMPARTMENT 4
  COMP= (7); TRANSIT COMPARTMENT 5
  COMP= (8); TRANSIT COMPARTMENT 6
  COMP= (9); ABSORPTION COMPARTMENT
  COMP= (10); AUC
```



```
$PK
  IF(NEWIND.LE.1) THEN
  COM (1) =-1
  COM (2) =-1
  ENDIF

  TVCL=THETA (1) + (THETA (5) *(CYPACTIVITY-100))
  CL=TVCL*EXP (ETA (1))
```

```
TVV=THETA (2)
V=TVV*EXP (ETA (2))

TVMTT=THETA (3)
MTT=TVMTT*EXP (ETA (3)) ; Mean transit time

KA=2.9
```

; First-order absorption rate constant was fixed to estimate population value of 2.9 h^{-1}.

```
S2=V/1000 ; Scaling factor

NN=6                    ; Number of transit compartments
KTR=(NN+1)/MTT      ;Transit rate constants
K13=KTR
K34=KTR
K45=KTR
K56=KTR
K67=KTR
K78=KTR
K89=KTR
K92=KA
K20=CL/V
HL=LOG (2)*V/CL ; model predicted elimination half-life

; BASELINE CONCENTRATION MODEL
C0=THETA (4) *EXP (ETA (4))          ; Predicted baseline concentration
TY=C0*EXP(-K20*TIME)                  ; Model for baseline concentration
```

```
$DES
   DADT (1) = – A (1) *K13
   DADT (2) = A (9) *K92 – A (2) *K20
   DADT (3) = A (1) *K13 – A (3) *K34
   DADT (4) = A (3) *K34 – A (4) *K45
   DADT (5) = A (4) *K45 – A (5) *K56
   DADT (6) = A (5) *K56 – A (6) *K67
   DADT (7) = A (6) *K67 – A (7) *K78
   DADT (8) = A (7) *K78 – A (8) *K89
   DADT (9) = A (8) *K89 – A (9) *K92
   DADT (10) = A (2)
   AUC= A (10)/S2

   CT= A (2)/S2
   IF (CT.GT.COM (1)) THEN
   COM (1) =CT
   COM (2) =TIME
   ENDIF
```



```
$ERROR
DEL=0
IF ((F+TY).LE.0.0001) DEL=1
 IPRE=F+TY
 W= IPRE +DEL
 IRES= DV-IPRE
 IWRE=IRES/W
   Y = IPRE + W *ERR (1)

CMAX = COM (1)
TMAX = COM (2)
```

$EST METHOD=1 INTERACTION PRINT=5 MAX=9999 SIG=3

$THETA

(0,250) ;[CL/F]

(0,800) ;[V/F]

(0, 0.1) ;[MTT]

(0, 0.5) ;[C0]

(2) ;[CYP2A6]

$OMEGA

0.04 ;[P] omega for CL/F

0.04 ;[P] omega for V/F

0.04 ;[P] omega for MTT

0.04 ;[P] omega for C0

$SIGMA

0.04 ;[P] sigma for proportional error

$COV

$TABLE ID CL V MT C0 CMAX TMAX HL AUC TIME ETA1 ETA2 ETA3 ETA4 PRED RES WRES IPRE IWRE CPRED CWRES AMT DV EVID MDV AGE WT HEIGHT IBW ABW BMI BSA CYPACTIVITY OBESE TWOPHENO SEVENGENO THREEGENO ALCOHOL YSMOKE YSMOKEC NSMOKE FTND ONEHEADER NOPRINT FILE=finalmodel.tab

VITA

NAME	Kathy Moe San
DATE OF BIRTH	28 Nov 1989
PLACE OF BIRTH	Pauk Township, Magway District, Myanmar
INSTITUTIONS ATTENDED	University of Pharmacy, Mandalay, Myanmar
HOME ADDRESS	Yandanar Thiri street, Pauk Township, Magway District, Myanmar